TECHNICAL REPORT 7509

PROBLEM DEFINITION STUDIES ON POTENTIAL ENVIRONMENTAL POLLUTANTS

II. PHYSICAL, CHEMICAL, TOXICOLOGICAL, AND BIOLOGICAL PROPERTIES OF 16 SUBSTANCES

December 1975

US ARMY MEDICAL BIOENGINEERING RESEARCH and DEVELOPMENT LABORATORY
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Aldrin	Carcinogenesis	Dieldrin
Amphibians	Chlorate (ClO_3^-) salts	Diisopropyl methylphosphonate (DIMP)
Analytical methods	Chlordane	Endrin
Arsenic compounds	Decomposition	Fish
Birds	Dicyclopentadiene (DCPD)	Invertebrates

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This report establishes a data base of physical, chemical, toxicological, and biological properties for: mustard gas; thiodiglycol; lewisite; lewisite oxide; methylphosphonic acid; isopropyl methylphosphonate; diisopropyl methylphosphonate; chlorate salts; wheat rust; inorganic arsenic compounds; mercury and its salts; dicyclopentadiene; aldrin; dieldrin; chlordane; and endrin, and provides a summary of pertinent information concerning: physical and chemical properties; analytical methods; mammalian toxicology; ecological considerations

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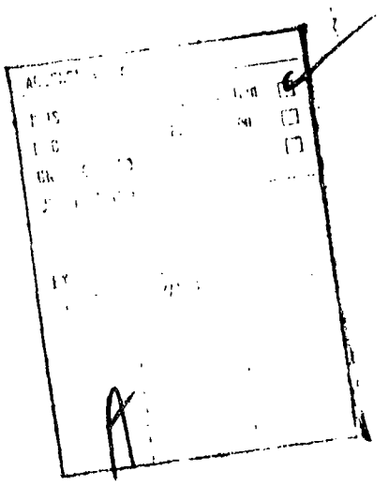
Isopropyl methylphosphonate (IMP)	Physico-chemical properties
Lewisite	Plants
Lewisite oxide	Pollution
Mammals	Reptiles
Man	Standards
Mercury and mercury salts	Teratogenesis
Metabolism	Thiodiglycol
Methylphosphonic acid (MPA)	Toxicity
Microorganisms	Translocation
Miscellaneous biological effects	Transport
Mustard gas	Volatilization
Mutagenesis	Wheat rust
Persistence	Wildlife

20. ABSTRACT (Cont'd)

for wildlife, birds, fish, reptiles, amphibians, invertebrates, microorganisms, and plants; and existing standards



PREFACE

This problem definition study was completed as the result of an intensive effort by a team organized at the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL). The team consisted of members of the professional staff of the Environmental Protection Research Division (EPRD), USAMBRDL, and professional consultants from Walden Research Division (WRD) of Abcor, Incorporated. Individuals whose professional expertise contributed significantly to the completion of this report are listed as contributors. Individuals who had primary responsibility for both the management of the team effort and the principal editing of this report are listed as editors.

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INTRODUCTION

An earlier report (1) assessed the toxicology and ecological hazards of the following 16 substances that were identified as potential environmental pollutants at Rocky Mountain Arsenal (RMA): mustard gas, thiodiglycol, lewisite, lewisite oxide, methylphosphonic acid, isopropyl methylphosphonate, diisopropyl methylphosphonate, chlorate salts, wheat rust, inorganic arsenic compounds, mercury and its salts, dicyclopentadiene, aldrin, dieldrin, chlordane, and endrin. That assessment included a discussion of the occurrence of these substances at RMA and their anticipated behavior in that milieu; the development of a rationale for the calculation of preliminary Soil Pollutant Limit Values (SPLV's) for those substances about which sufficient information was available; and the identification of information voids and recommendations for research to supply information needed to adequately assess adverse health and environmental effects. The basis for studying these particular substances, the organization of the technical and professional personnel who conducted the study, the manual and computerized literature searches conducted, and the information handling system that was used are detailed in that earlier report.

OBJECTIVE

The objective of this report is to provide a data base of physical, chemical, toxicological, and biological properties of the 16 potential environmental pollutants that were addressed in the earlier report (1).

SUMMARY OF FINDINGS

The findings from this study are presented in detail for each pollutant substance in Appendixes A through M. Pertinent information concerning physical/chemical properties, analytical methods, mammalian toxicology, ecological considerations, and existing standards has been extracted from the appendixes and is summarized in Tables 1-6.

Table 1 groups the pollutants according to volatility, water solubility, and potential for waterborne movement through soil. Predictions of waterborne movement are based on available information concerning solubility and chemical stability in water. Selected physical properties of the pollutant substances are summarized in Table 2.

The summary of analytical methods (Table 3) is not exhaustive or definitive. Analytical laboratories tend to use standard procedures and available instrumentation. Generally, gas chromatography with suitably specific and sensitive detectors can be used for mustard gas, thiodiglycol (low sensitivity), lewisite, diisopropyl methylphosphonate, aldrin, dieldrin, chlordane and endrin. Conversion to appropriate derivatives should permit gas chromatographic analysis of lewisite oxide, isopropyl methylphosphonate, and methylphosphonic acid. Arsenic and mercury can be determined by flameless atomic absorption or neutron activation analysis (methods presumably adaptable to lewisite and lewisite oxide); there are also sensitive chemical methods available for arsenic and mercury. Chlorate ion is unique in that only chemical (colorimetric) methods are suitable for its analysis; care must be taken to rule out other oxidants with similar effects on the colorimetric reagents.

The important mammalian toxicological properties of each pollutant substance are summarized in Table 4.

Ecological considerations for each pollutant substance are summarized in Table 5 according to various animal groups, microorganisms, plants, and food chain effects. Where these categories are not included in the summaries, ecological effects were not found in the literature.

Standards pertaining to Allowable Daily Intake (ADI), Maximum Contaminant Levels (MCL), and Threshold Limit Values (TLV) for the pollutant substances are summarized in Table 6. Detailed information concerning the status of these values, or qualifications placed on them, are presented in the footnotes to Table 6, and the supporting references, and are not repeated here.

TABLE 1. SUMMARY OF POLLUTANTS GROUPED ACCORDING TO VOLATILITY, WATER SOLUBILITY, AND POTENTIAL FOR WATERBORNE MOVEMENT THROUGH SOIL[a]

Volatility	
Essentially non-volatile:	Salts of arsenic; salts of mercury; chlorate ion; isopropyl methylphosphonate ion; methylphosphonate ion; oxides of arsenic and mercury; lewisite oxide (polymer form)
Very low volatility:	Thiodiglycol; monomers of lewisite oxide; some arsenic and mercury compounds; aldrin; dieldrin; chlordane; endrin; methylphosphonic acid; isopropyl methylphosphonate; diisopropyl methylphosphonate
Low volatility:[b]	Mustard gas; lewisite; dicyclopentadiene; metallic mercury
Moderate to high volatility:	Alkylated forms of mercury and arsenic; arsine
Solubility in Water	
Very low solubility: (<200 ppm)	Oxides and some salts of arsenic and mercury; aldrin; dieldrin; chlordane; endrin
Solubility with decomposition:	Mustard gas (slow) and lewisite (fast)
Slight solubility: (200-20,000 ppm)	Lewisite oxide; diisopropyl methylphosphonate; some arsenic and mercury salts
Moderate to high solubility:	Methylphosphonic acid and its salts; isopropyl methylphosphonate and its salts; some chlorate, arsenic, and mercury salts
Waterborne Movement Through Soil	
Very low mobility:	Aldrin; dieldrin; chlordane; endrin; mustard gas (owing to decomposition); lewisite (owing to decomposition); some forms of mercury, arsenic, and lewisite oxide
Low to moderate mobility:	Some forms of lewisite oxide, mercury and arsenic; dicyclopentadiene; diisopropyl methylphosphonate
High mobility:	Thiodiglycol; methylphosphonic acid and its salts; isopropyl methylphosphonate and its salts; chlorate salts; some forms of mercury and arsenic

a. Wheat rust is not included in this summary.
b. Low volatility significant under certain circumstances.

TABLE 2. SUMMARY OF SELECTED PHYSICAL PROPERTIES OF POLLUTANTS[a]

Pollutant	Melting Point (°C)	Boiling Point (°C)	Density (g/ml)	Solubility in Water (Typical Values)
Mustard gas	14.4	228	1.27	0.1% with rapid hydrolysis to thiodiglycol
Thiodiglycol	-10	164/20 mm	1.22	Infinite
Lewisite	-1.2	170	1.86	Instant hydrolysis to lewisite oxide
Lewisite oxide	140 (polymer)	--[b]	--[b]	1%
Methylphosphonic acid	107	--[b]	--[b]	High
Isopropyl methyl-phosphonate	Liquid	98/0.08 mm	1.11	Probably high; very slow hydrolysis
Diisopropyl methyl phosphonate	Liquid	174	0.98	0.1-0.2%
Chlorate salts[c]	Solid salts	--	--	Soluble
Arsenic[c]	--	--	--	--
Mercury[c]	--	--	--	--
Dicyclopentadiene	32.9	166.6	0.98	40 ppm[d]
Aldrin	104	--[b]	--[b]	0.027 ppm
Dieldrin	175	--[b]	--[b]	0.19 ppm
Chlordane	--[b]	175/2 mm	1.6	<0.1 ppm[e]
Endrin	235[d]	--[b]	1.65	0.23 ppm

a. Wheat rust is not included in this summary.
b. Values not determined in this literature search.
c. Values vary with specific compound.
d. Value estimated.
e. By inference from reference 2.

TABLE 3. SUMMARY OF ANALYTICAL METHODS MOST APPLICABLE TO CHEMICAL POLLUTANTS[a]

Pollutant	Analytical Preferred Methods
Mustard gas	Gas chromatography with electron capture detector (FID detector has been used); 4-(p-nitrobenzyl) pyridine colorimetric test
Thiodiglycol	Gas chromatography with FID detector or flame photometric sulfur detector
Lewisite	Atomic absorption; gas chromatography; Gutzeit test via arsine
Lewisite oxide	Atomic absorption; gas chromatography (after derivatization); Gutzeit test via arsine
Methylphosphonic acid	Gas chromatography after esterification; paper chromatography; electrophoresis
Isopropyl methyl-phosphonate	Gas chromatography (preferably after esterification)
Diisopropyl methyl-phosphonate	Gas chromatography with flame photometric phosphorus detector
Chlorate salts	Colorimetry based on oxidation of organic compounds at low pH; thin-layer or paper chromatography
Arsenic	Flameless atomic absorption; neutron activation; colorimetry (arsenomolybdate or silver diethyldithiocarbamate methods); Gutzeit test via arsine
Mercury	UV absorption of metallic Hg vapors; flameless atomic absorption; neutron activation; colorimetry (dithizone)
Aldrin, dieldrin, chlordane, endrin	Gas chromatography with electron capture or microcoulometric detectors

a. Wheat rust is not included in this summary; see Appendix G for analytical methods.

TABLE 4. SUMMARY OF TOXICOLOGICAL PROPERTIES OF POLLUTANTS

Pollutant	Toxicological Properties
Mustard gas	Highly irritant; skin sensitizer; mutagenic; carcinogenic to animals and man
Thiodiglycol	Acute toxicity 4 to 6.6 g/kg; toxicity presumed similar to glycols; toxicology otherwise unknown
Lewisite/lewisite oxide	Highly irritating; decomposition products presumed similar in toxicity to arsenic compounds; toxicology otherwise unknown
Methylphosphonic acid	Toxicology unknown
Isopropyl methylphosphonate	Toxicology unknown
DIMP	Rabbit i.v. LD_{50} 224 mg/kg; not a skin irritant; toxicology otherwise unknown
Dicyclopentadiene	Mildly irritant in animals; moderately toxic by inhalation to animals; carcinogenicity test (intramuscular in rats) negative; toxicology otherwise unknown
Chlorate salts	Lethal dose to man is 5-30 gm; severe hemolytic anemia in dogs fed 200 mg/kg/day; toxicology otherwise unknown
Wheat rust	Toxicology unknown
Arsenic compounds	Skin disorders; abnormal pigmentation; carcinogenic in man; possible teratogen in animals; lethal in small single doses
Mercury compounds	Neuro toxic; mild skin sensitizer; mutagenic; teratogenic in animals and man; postnatal development abnormalities in animals and man; repeated low level exposure is highly toxic in man
Aldrin/dieldrin	Single lethal dose in man is ~ 5 grams; repeated doses of 0.5 mg/man/day no effect; carcinogenic in mice; not in rats; possibly teratogenic in animals; mutagenicity not demonstrated

TABLE 4 (cont'd)

Pollutant	Toxicological Properties
Endrin	Toxic to man at 0.3-3 grams; highly toxic in single doses in animals; carcinogenicity hazard uncertain, but so far negative; accumulation in fat not significant; possibly teratogenic in animals
Chlordane	Single lethal dose in man is ~ 5 grams; industrial human exposure had no appreciable effect; carcinogenic in mice, not in other species; accumulation in fat not significant

TABLE 5. SUMMARY OF ECOLOGICAL CONSIDERATIONS FOR POLLUTANT SUBSTANCES

Pollutant Substance	Ecological Considerations
Mustard gas	Invertebrates: mutagenic to fruit fly; 0.01% used as bean beetle insecticide Microorganisms: mutagenic at 6.0×10^{-4} to 5.0×10^{-3} M; primary toxic effects prevention of total DNA replication because of inter-strand crosslinkage at guanine bases; lethal to E. coli at 0.8 to 6.0 mg/ml Plants: toxic to leaves, roots, and embryo; tolerated in small doses; mutagenic or produces sterility
Thiodiglycol	Plants: no herbicidal effects
Lewisite/lewisite oxide	Fish: toxic doses from 0.2 to 2.0 ppm; non-toxic after 50 days in water Amphibians: toxic at 0.5 ppm to tadpoles Plants: apparently phytotoxic as liquid or vapor Food Chain: little chance for bioaccumulation
Methylphosphonic acid	Plants: hormone-like effects; severe contact injury and stunting at 0.1 to 10 lb/acre; lethal to aquatic plants at 1000 ppm and algae at 100 ppm
Isopropyl methylphosphonate	No information available
Diisopropyl methylphosphonate	Plants: leaf edge burn on wheat and bean leaves at 10 and 40 ppm; phytotoxic to sugar beets

TABLE 5 (cont'd)

Pollutant Substance	Ecological Considerations
Chlorate salts	Fish: lethal to fish above 1000 ppm; lethal to plankton above 100 ppm Microorganisms: lethal to coliform bacteria at 0.002%; lactic acid bacteria less sensitive; numerous species tolerate 1% solutions; can be reduced by soil microbes to toxic chlorite in presence of nitrate Plants: sensitivity depends on plant species and soil type; stunted growth; toxic range 6-1000 ppm; systemic poison that is cumulative until cell death; reduced in plant to toxic chlorite; can interfere with nitrate metabolism Food Chain: no evidence for accumulation or transport in ecosystem
Wheat rust	Plants: removes photosynthate needed for wheat growth; needs specific host (barberry) for overwintering in cold climates
Arsenic	Birds: toxicity depends on arsenic compound and bird species Amphibians: toxicity ranges for tadpoles from 130 to 910 mg/l (30-600 min), depending on compound and length of exposure Fish: toxicity ranges from 3.1 to 11.6 mg/l, depending on fish species and length of exposure Invertebrates: toxicity ranges from 3 to >361 mg/l, depending on species Microorganisms: toxicity varies among species from 290 to 10^4 mg/l; more toxic to some species when little phosphate present; enters phosphate transport system; reduced by many species anaerobically

TABLE 5 (cont'd)

Pollutant Substance	Ecological Considerations
Arsenic (cont'd)	Plants: toxicity depends on availability in soil, plant species, soil content, pH, and chemical form; causes 50% growth reduction at 6.2 to 48.3 ppm available arsenic in some plants; uptake depends on amount available; more in roots than tops; concentrates to 76 ppm in radishes grown on soil containing 19 ppm; aquatic plants concentrate 100 to 71,000 X Food Chain: accumulated by aquatic plants and animals from the water and by a few terrestrial plants from soil; not concentrated through food chain
Mercury and mercury salts	Birds: methylmercury toxic at 3.9-10 ppm in food; mercury concentrates to levels above ambient intake; mercuric nitrate causes decreased hatchability and is passed to the egg Amphibians: high mercury levels in amphibians from mercuriferous areas Fish: bioaccumulates 10^3 X from water; produces behavioral changes at 0.003 ppm; accumulates in fish muscle, not vital organs; percent deposition increases with age of fish; retention varies with species, with 200 to >600 days required to excrete 1/2 of ingested mercury Invertebrates: mutagenic to fruit flies at 0.25 ppm; bioaccumulates 10^5 X from water Microorganisms: used as a bactericidal agent with variable toxicity; toxic to some species initially at 0.1 to 0.5 g mercuric chloride/ 100 g soil, but tolerance develops; $HgCl_2$ metabolized to CH_3Hg^+ by some species; Hg metabolized to metallic Hg or HgS; resistance a function of mercuric ion-reducing enzyme; levels above 5 to 40 ppm inhibit growth

TABLE 5 (cont'd)

Pollutant Substance	Ecological Considerations
Mercury and Mercury Salts (cont'd)	Plants: toxicity varies with plant species; vapor injurious; monocots appear to be more resistant than dicots; reduces photosynthesis in phytoplankton at less than 0.1 ppb; absorbed through leaves and roots; translocated to leaves and fruit; accumulated from 6-1087 ppm in edible portions of plants grown in 10 ppm mercuric chloride in soil Food Chain: bioconcentrates in aquatic food chains, theoretically up to 10^{11} X in large fish
Dicyclopentadiene	Plants: causes leaf tip burn at 10 and 40 ppm
Aldrin/Dieldrin	Birds: acute oral LD_{50} from 6.6 to 520 mg/kg; lowest daily tolerated dose (30 days) 1.25 to 5 mg/kg; reproductive impairment (low hatchability, decrease shell thickness, decrease fertility) at 1 to 50 ppm Fish: fish extremely sensitive to aldrin and dieldrin; lethal at .0075 to 0.32 ppm for 96 hours; causes behavioral changes and reproductive impairment at less-than-lethal levels Reptiles: accumulates in fat of turtles Invertebrates: toxic in ppb range to crayfish (aldrin); 0.1 to 1 ppm dieldrin interferes with shell deposition and feeding behavior of oysters; earthworms concentrate 2-10 X from soil; bees very sensitive (LD_{50} = 0.149 µg/bee) Microorganisms: toxicity dependent on temperature, pH, clay and organic content; 200 lb/acre depress ammonia or sulfur oxidation; aldrin metabolized to dieldrin and diol; dieldrin metabolized to photodieldrin and diol; basic ring structure attacked

TABLE 5 (cont'd)

Pollutant Substance	Ecological Considerations
Aldrin/Dieldrin (cont'd)	Plants: inhibits root development and germination; decreased yields; aerial and root absorption dependent on plant species, soil type, concentration, and depth; accumulates mostly in epidermis of root crops; translocated; aldrin degraded to dieldrin (70-80%); dieldrin degraded (perhaps) to alcohols and ketones Food Chain: aldrin converted to dieldrin in food chain; plants and worms bioaccumulate from soil; snails, fish and algae bioconcentrate 1000 X from water; potential contamination of higher trophic levels
Chlordane	Birds: toxic at 2% spray Fish: 96 hr LC_{50} from 1 to 2,000 µg/l, depending on species Invertebrates: 24 hr LC_{50} 100 to 120 µg/l for freshwater invertebrates; accumulates in earthworms Microorganisms: 500 lg/acre reduces numbers of soil fungi and nitrifying bacteria; 200 lb/acre depresses ammonia and sulfur oxidation in soil; interferes with oxidative metabolism, utilized by Aspergillus niger Plants: sensitivity depends on plant species; 65 lbs/acre reduce Kentucky blue grass stand 95%; toxic to tomatoes at 400 lbs/acre and melons at 25 lbs/acre; accumulates primarily in roots; 9.6% of soil chlordane present in sugar beets; metabolized in plant to cis-, trans-, photo-cis-, and oxychlordane
Endrin	Birds: lethal to pheasants at 5 ppm in food Fish: fish very sensitive; 96-hour LD_{50} from 0.6 ppb to 1.8 ppb for adults

TABLE 5 (cont'd)

Pollutant Substance	Ecological Considerations
Endrin (cont'd)	Invertebrates: reduces soil invertebrate populations; earthworms concentrate 3.6 times Microorganisms: not lethal at 5 lbs/acre for 5 years; metabolized by soil and marine sediment microflora to ketoendrin Plants: causes retarded growth; inhibited flowering, and leaf burn; alters N and P uptake of wheat and corn; uptake by leaves and roots; accumulation in root crops; uptake dependent on plant species, soil type, and concentration; metabolized to endrin ketone and endrin alcohol Food Chain: aquatic organisms may bioaccumulate 10^2 to 10^4 times from water; dietary levels reflected in cow's milk

TABLE 6. SUMMARY OF EXISTING STANDARDS FOR POLLUTANTS

Pollutant	Allowable Daily Intake (ADI) (mg/kg/day)	Maximum Contaminant Level (MCL) in Water (mg/l)	Threshold Limit Value (TLV) (mg/m^3)
Mustard gas	--	--	$4x10^{-3}$ j
Thiodiglycol	--	--	$2.5x10^{2}$ k
Lewisite/lewisite oxide[a]	--	2[f]	--
Chlorate salts	None[b]	--	--
Arsenic compounds	$5x10^{-2}$ c	$5x10^{-2}$ g	$5x10^{-1}$ l
Mercury compounds	$7.1x10^{-4}$ d $4.7x10^{-4}$ (methyl Hg)	$2x10^{-3}$ g	$5x10^{-2}$ l $1x10^{-2}$ (alkyl Hg)
DCPD	--	--	23 l
Aldrin	$1x10^{-4}$ e	$1x10^{-3}$ g	$2.5x10^{-1}$ l
Chlordane	$1x10^{-3}$ e	$3x10^{-3}$ h	$5x10^{-1}$ l
Dieldrin	$1x10^{-4}$ e	$1x10^{-3}$ i	$2.5x10^{-1}$ l
Endrin	$2x10^{-4}$ e	$2x10^{-4}$ g	$1x10^{-1}$ l

See following page for footnotes.

FOOTNOTES FOR TABLE 6

a. Lewisite is rapidly converted to lewisite oxide upon exposure to environmental moisture; values for lewisite are considered as applying to lewisite oxide also.

b. Potassium chlorate is regarded as too toxic to allow its use as a food additive (reference 3).

c. See reference 4.

d. Weekly values cited in reference 5 were divided by seven. These are "provisional tolerable weekly uptake" values.

e. See reference 6.

f. A personal communication concerning reference 7 indicates that the value is in terms of arsenic, and is restricted to 1 week of such supply under emergency battlefield conditions.

g. These are maximum contaminant levels (MCL's) values for arsenic, mercury and endrin in drinking water (reference 8).

h. This is a proposed value for chlordane and is still under review by the USEPA (reference 9).

i. These are proposed values for aldrin and dieldrin in raw water as recommended by the National Academy of Sciences, and are still under review by the USEPA (reference 10).

j. The sulfur mustard (mustard gas) data were incorrectly summarized in reference 11; they have been corrected here.

k. Estimated value (see reference 1).

l. Except for arsenic, other values are for skin adsorption exposure (reference 12).

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APPENDIX A

MUSTARD GAS/THIODIGLYCOL

Mustard gas undergoes hydrolysis to thiodiglycol. Hence, these two compounds are discussed together in this appendix.

ALTERNATIVE NAMES

MUSTARD GAS: Sulfur mustard; mustard; Levinstein mustard; ethane, 1,1'-thiobis(2-chloro)- (Chem. Abstr. after 1971); sulfide, bis(2-chloroethyl) (Chem. Abstr. through 1971); yperite.

THIODIGLYCOL: Ethanol, 2,2'-thiodi- (Chem. Abstr. 1937-1971); bis (β-hydroxyethyl) sulfide; bis(2-hydroxyethyl sulfide); β β'-dihydroxydiethyl sulfide; β, β'-dihydroxyethyl sulfide; β-hydroxyethyl sulfide; Kromfax solvent; 2,2'-thiodiethanol; thiodiethylene glycol; β-thiodiglycol; ethanol, 2,2'-thiobis- (Chem. Abstr., before 1937 and after 1971).

PHYSICAL AND CHEMICAL PROPERTIES

MUSTARD GAS:
CAS Reg. No. 505-60-2
Defense Department symbols: H, HD
Toxic Substances List: KI92750
Edgewood Arsenal Number: EA 229
Wiswesser Line Notation: G2S2G
Molecular formula: $C_4H_8Cl_2S$

Structural formula: $Cl\text{-}CH_2CH_2\text{-}S\text{-}CH_2CH_2\text{-}Cl$

THIODIGLYCOL:
CAS Reg. No. 111-48-8
Toxic Substances List: KM29750
Edgewood Arsenal Number: EA 1019
Wiswesser Line Notation: Q2S2Q
Molecular formula: $C_4H_{10}O_2S$

Structural formula: $HOCH_2CH_2\text{-}S\text{-}CH_2CH_2OH$

Mustard gas was first synthesized by the physician Francais Despretz (1) in 1822; however, its toxicity was not discovered until 1860 through the independent observations of A. Neimann (2) and F. G. Guthrie (3). Its first military use, by Germany in World War I at Ypres (June 1917), caused mass casualties (4). The Allied powers adopted this chemical agent and produced it efficiently in large amounts, so that it became the principal toxic agent in the last year of the War. The Italians used mustard gas in their campaign against Ethiopia in the 1930's (3), but the agent was not employed in World War II.

Three processes have been used in the manufacture of mustard gas:

a. V. Meyer process, used by the Germans (5, 6):

$$2\ ClCH_2CH_2OH + Na_2S \rightarrow S(CH_2CH_2OH)_2 \xrightarrow{2HCl} S(CH_2CH_2Cl)_2 + 2H_2O$$

b. Levinstein process (5, 7):

$$2\ CH_2{=}CH_2 + S_2Cl_2 \longrightarrow S(CH_2CH_2Cl)_2 + S$$

c. Most recent American process (8, 9):

$$2\ CH_2\text{-}\underset{O}{\text{}}\text{-}CH_2 + H_2S \rightarrow S(CH_2CH_2OH)_2 \xrightarrow{2HCl} S(CH_2CH_2Cl)_2 + 2\ H_2O$$

The Levinstein process, which was used for some time by the British and Americans, produces a complex mixture that includes some constituents more toxic than mustard gas itself (10, 11). Part of the sulfur formed in the Levinstein process reaction is not the free element, but is combined in various bis-(2-chloroethyl) polysulfides.

Mustard gas can be generated rapidly for small-scale use through the reaction of either boron trichloride or concentrated hydrochloric acid with thiodiglycol (12).

Some of the physical properties of mustard gas and thiodiglycol are summarized in Table A-1.

The following properties of mustard gas and (for the most part) of thiodiglycol at 20° (or 25°), along with some equations for temperature dependence, have been listed by Moelwyn-Hughes (15): Density, refractive index, molar refraction, viscosity, surface tension, parachor, vapor pressure, ebullioscopic constant, cryoscopic constant, freezing point, latent heat of vaporization, and specific heat.

TABLE A-1. Selected Physical Properties of Mustard Gas and Thiodiglycol

	Mustard Gas (13)	Thiodiglycol (14)
Molecular weight	159.08	122.19
Melting point, °C	14.4	-10
Boiling point, °C	228	164 (20 mm Hg)
Flash point, °C	105	--
Vapor pressure (20°C), mm Hg	0.72	--
Heat of vaporization, Kcal/mol	15.0	--
Heat of fusion, Kcal/mol	4.3	--
Heat of combustion, Kcal/mol	708	--
Heat of formation, Kcal/mol	32	--
Viscosity (20°C), poise	0.046	--
Liquid density (20°C), g/cc	1.27	1.22
Specific heat, liquid, cal/g-°C	0.330	--

An empirical vapor pressure - temperature relation for mustard gas was reported in 1932 by Mumford, et al. (16) as: Log P(mm Hg) = 8.3937 - 2734.5/T(°K), while in a 1948 article by Redemann, et al. (17), the equation Log P (mm Hg) = 9.31768 - 3062.5/T(°K) was given. Information is available on the compressibility of mustard gas and the change of melting point with pressure (18). The thermal decomposition of mustard gas has been studied, with products identified (19) and disappearance rates determined (13).

Mustard gas has a solubility in water of about 0.07% (20, 21), and with temperature increases slightly in solubility (22) - i.e., 0.075% at

10^0 and 15^0, 0.081% at 20^0 and 0.104% at 30^0. From the following equation (23), one can calculate the solution rate of mustard gas in distilled water:

$$S = 233.7 \times e^{-12,350/RT}$$

Thus, at 10°C, the rate is calculated at 6.77×10^{-8} g cm^{-2} sec^{-1}. Because of the very slow rate of solution of mustard gas in water, this compound is difficult to decontaminate by hydrolysis despite the relatively high reaction rate constants involved in hydrolysis (see below). Various aqueous organic solvents dissolve mustard to a considerable extent (24). The miscibility of mustard gas with various organic solvents has been determined (2). Thiodiglycol is considered infinitely water soluble (14).

Mustard gas readily undergoes both hydrolysis and oxidation. The hydrolysis involves several pathways (25, 26), as illustrated in Figure A-1. The top row reactions normally occur when mustard hydrolyzes in the presence of large amounts of water, whereas conditions involving relatively small quantities of water give rise to intermediates such as II, III and IV, which are rather toxic. Some rate studies covered a wide range of temperatures (20, 22, 27).

Very careful work by Bartlett and Swain (28) established values at 25°C of $k_1 = 0.155$ min^{-1} and $k_1' = 0.260$ min^{-1} (See Figure A-2). Although values of k_w, k_w, k_{-1}, k_{-1}, k_2 and k_2' cannot be determined, it is possible to measure competition factors, i.e., $k_2/k_w = F_{X^-}$. A nucleophilic reagent, X^-, with a high competition factor causes the product $ClCH_2CH_2S\text{-}CH_2CH_2X$ to be formed in preference to $ClCH_2CH_2S\text{-}CH_2CH_2OH$ although the rate of disappearance of mustard gas is normally not affected. If the competitor is Cl^- (Figure A-2), the overall result is to slow the observed rate of mustard gas decomposition; that is, mustard gas is formed again by back reaction of the cyclic intermediate with chloride ion. Here,

$$\frac{\text{Hydrolysis rate in presence of } Cl^-}{\text{Hydrolysis rate in water}} = \frac{1}{1 + F_{Cl^-}[Cl^-]}$$

Thus, the hydrolysis of mustard gas at 25°C is calculated to be 2.5 times as fast in fresh water as in sea water (29). Competition factors for a large number of nucleophiles are available (26, 30).

The normal hydrolysis reaction involves nucleophilic displacement of chloride ion through a first-order process, as described above, but a second-order beta-elimination of hydrogen chloride can take place in appropriate solvents, at high concentrations of hydroxyl ion, to give first chloroethyl vinyl sulfide (31), and then divinyl sulfide (31, 32 33), a product devoid of vesicant properties (32) but still somewhat toxic (31).

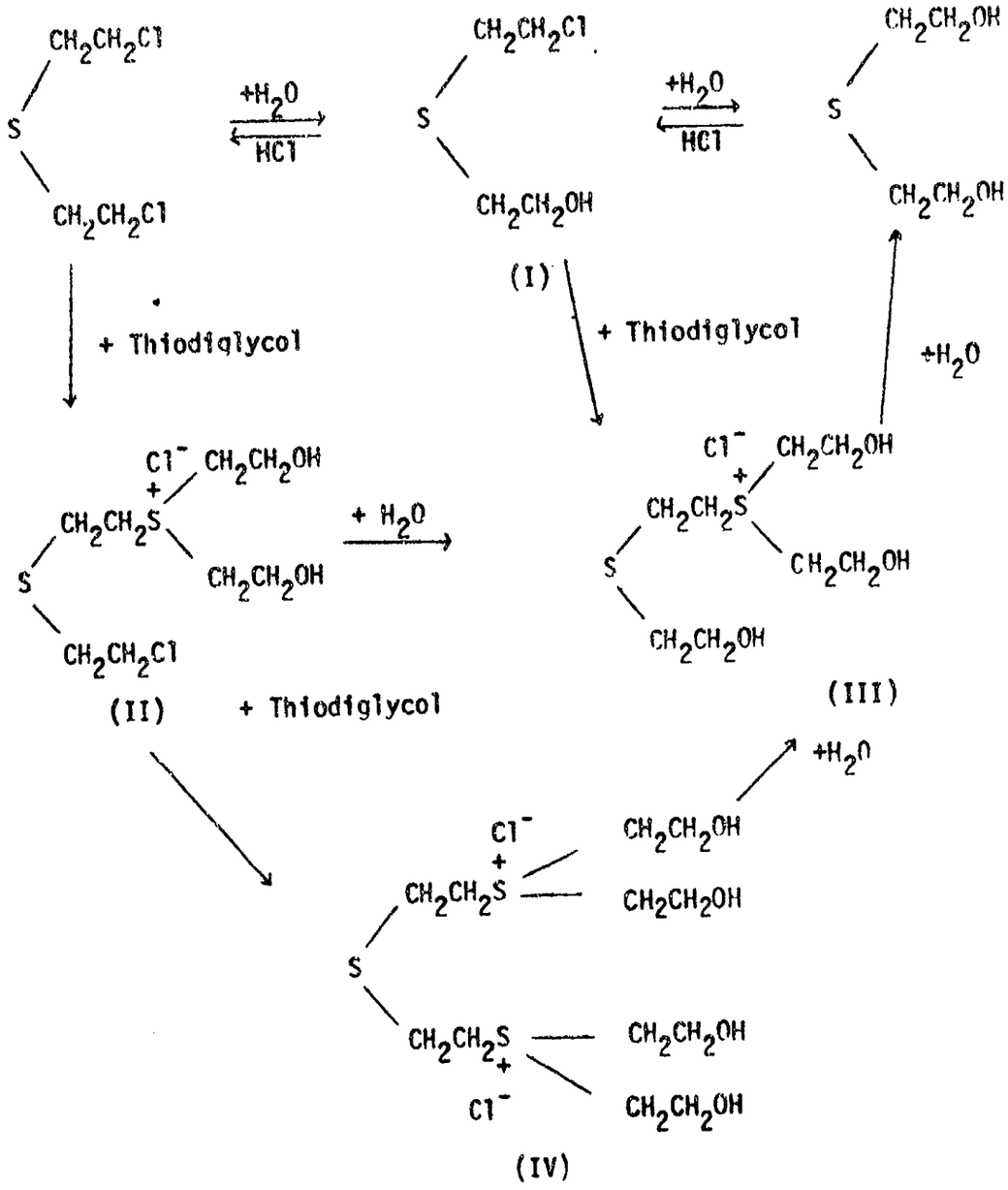


FIGURE A-1. Hydrolysis Pathways of Mustard Gas

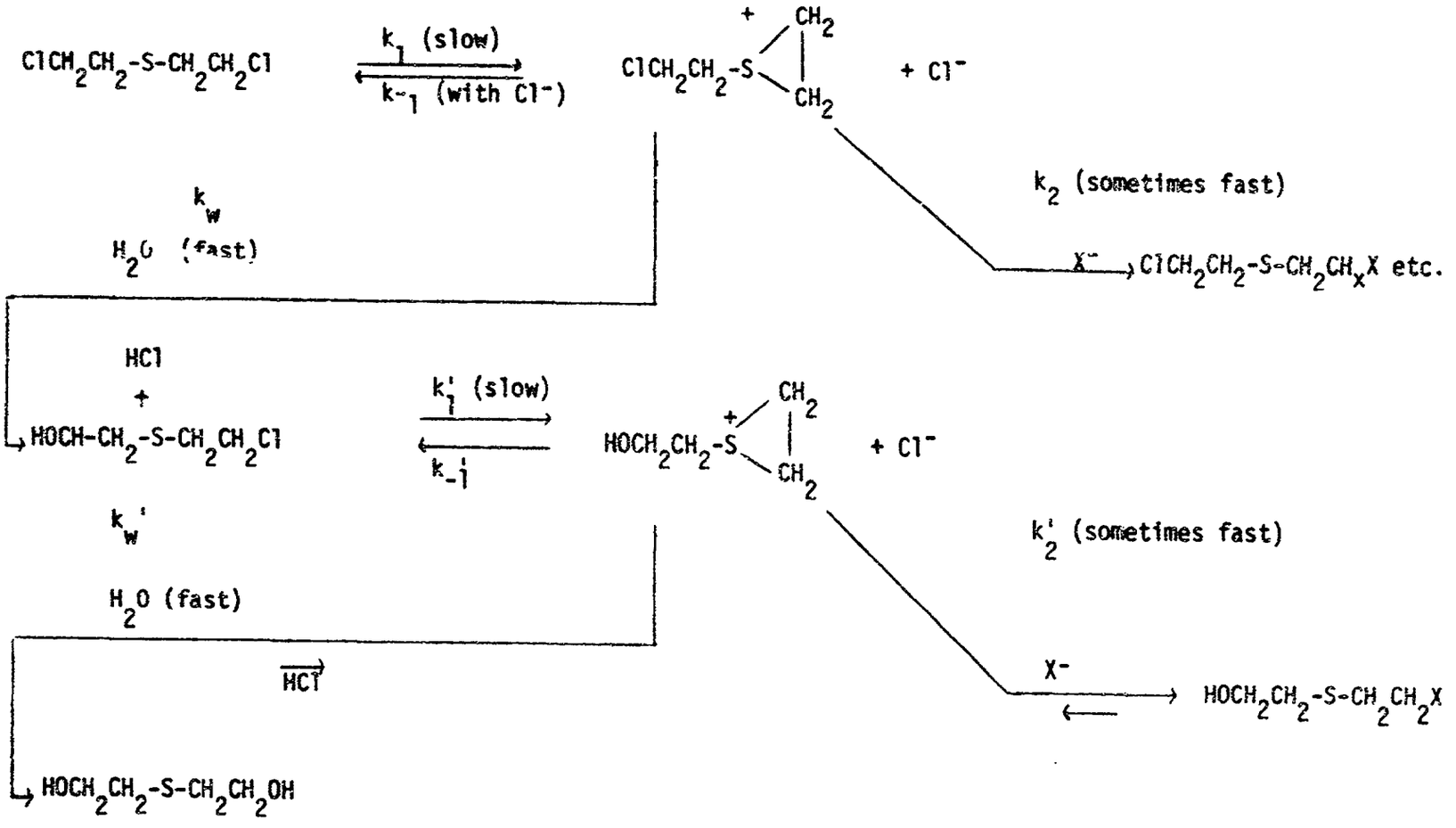
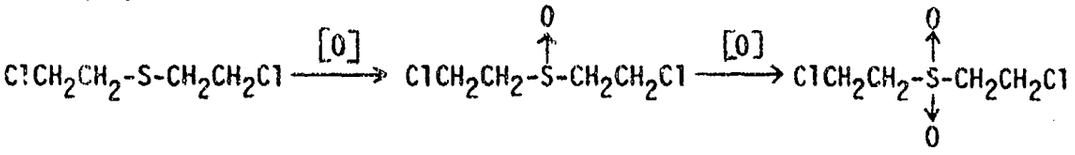


FIGURE A-2. Hydrolysis of Mustard Gas in the Presence of Chloride Ion

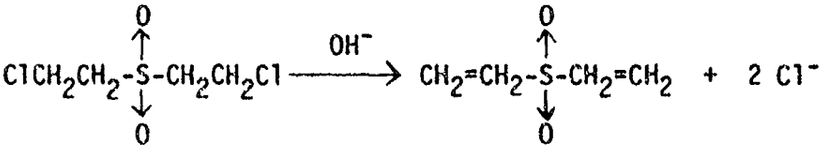
Exposure of mustard gas in aqueous solution to such oxidants as hypochlorites, chlorine water, ozone and hydrogen peroxide causes oxidation to the slightly toxic sulfoxide, which is extremely stable to hydrolysis, and then to the sulfone, which is slightly toxic but vesicant (34).



Mustard gas | Mustard Sulfoxide | Mustard Sulfone

Apparently the rate of oxidation with hypochlorite increases as oxidation proceeds, so that oxidation products past the sulfone are readily formed (35); reaction rates increase as the pH drops. The sulfoxide and sulfone oxidation products of thiodiglycol are considered nontoxic (34).

The sulfone, in weakly alkaline solution is dehydrochlorinated to divinyl sulfone, which is highly toxic intramuscularly, and extremely lachrymatory, but innocuous by ingestion at a concentration of 100 mg/l (2, 34):

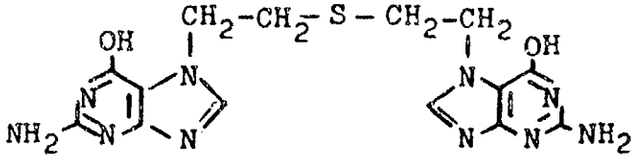


Mustard Sulfone | Divinyl Sulfone

Mustard gas is decontaminated by means of extensive oxidation or by dehydrochlorination. In practice, oxidation always involves some form of active chlorine. Examples include hypochlorite salts, such as chlorinated lime slurries (36, 37, 38, 39, 40, 41, 42) and N-chloroamides (36, 37, 39, 42). Other reactive oxidants are concentrated or fuming nitric acid, potassium permanganate and chromic acid (2); ceric sulfate and several peroxymetallic acids or salts, notably peroxytitanyl nitrate, are effective decontaminants (43), as is hydrogen peroxide (44). Two decontaminating solutions containing amines and alkali metal hydroxides, namely DS-2 and CD-1, dehydrochlorinate

mustard gas to divinyl sulfide, and the reaction kinetics of these formulations with the toxic agent have been studied in detail (45).

The chief biochemical (i.e., toxic) effects exerted by mustard gas are ascribed to chromosome injuries (i.e., mutagenic effects) brought about through modifying or cross-linking of the nucleic acid purines guanine and adenine (36, 46). An example is the cross-linking of guanine moieties (36):



For further discussion of mutagenicity, see "HUMAN TOXICOLOGY, *Experimental Animals.*"

Mustard gas shows its strongest antienzymic activity against hexokinase which regulates carbohydrate metabolism, and a weak anticholinesterase action (36).

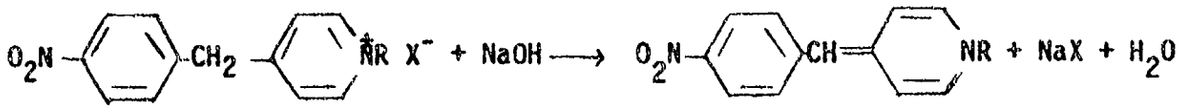
ANALYTICAL METHODS

Mustard is not normally found in aqueous solution because it is so easily hydrolyzed. This fact minimizes the need for detection or analysis of mustard in water, and makes research on such methods difficult.

Detection in air often takes the form of drawing relatively large volumes of air through small tubes or over paper containing silica gel impregnated with a suitable reagent. These reagents include chlorauric acid ($HAuCl_4$), (47, 48), platinum chloride, palladium chloride or cuprous chloride (48), or 4-(p-nitrobenzyl)pyridine with suitable metal salts (49, 50, 51, 52). The sensitivities of these tests vary somewhat, about 10 µg of mustard gas being detectable with chlorauric acid and about 0.5 µg by the 4-(p-nitrobenzyl)pyridine test.

The detection of mustard gas by the latter test is based on two reaction steps. In step one, the mustard gas, as represented by RX and preferably at 100°, reacts as an alkylating agent with 4-(p-nitrobenzyl) pyridine:

In step two, the addition of an alkaline solution immediately produces an intensely blue-colored dyestuff.



In other tests, contaminated air is bubbled through solutions of the reagents, for example 2,6-dichlorophenol-indophenol (53), sodium iodoplatinate without or with starch (49, 54, 55, 56), copper sulfate-sodium iodide (36), and two-step color formation with thiourea and nickel salts (57). These are, in general, less sensitive than the test with 4-(p-nitrobenzyl)pyridine.

Thin-layer chromatography on silica gel G has been used to separate and identify mustard gas, its monohydrolysis product ($ClCH_2CH_2$-S-CH_2CH_2OH) and thiodiglycol (58). The solvent is chloroform-acetone (50/40), and the R_f values are 0.80, 0.60 and 0.33, respectively.

A variety of titrimetric methods have been used in the past for the qualitative analysis of mustard gas, but are now mainly of historical interest. One of these is the reaction with a known amount of thiosulfate ion to displace -Cl with $-SSO_3^-$; the latter group is not reactive with triiodide ion, which is used to titrate excess thiosulfate.

Oxidative titrations, employing dichloramine-T (59), chloramine-T (60), bromine (60), or iodate (60), are equally applicable to mustard and thiodiglycol.

Colorimetric or spectrophotometric determinations of mustard gas have been somewhat more useful. 8-Quinolinol forms a color suited to such determinations (61). The iodoplatinate reaction (60), sensitive to about 5 µg when starch is added, is believed to involve mainly the following general reaction:

$$PtI_6^{=} + 4\ R_2S \rightarrow 4I^- + I_2 + Pt(R_2S)_4^{++}$$

The 4-(p-nitrobenzyl)pyridine test described above, used colorimetrically, is by far the most sensitive wet test, applicable down to 0.8 µg/l in 6.5 ml of sample (13, 62, 63). An improved bubbler for air sampling has been described in connection with this reaction (64).

Gas-liquid chromatography, by direct air-sample injection or by use of bubblers or extraction (for soil or vegetation) for sampling is today the method of choice for low-level analysis of mustard gas. Thus, samples

containing 0.16 μg/ml of mustard (13) or 0.2 μg/ml (65, 66) for a 1-μl injection can be analyzed when an electron-capture detector is used (13), and the limit is perhaps ten times lower for clean systems (13). Detection limits are somewhat higher with flame-ionization detectors (67). Analyses at 1 part per billion are projected for physiological samples (68). With 2-hr bubbler sampling, analysis of air containing as little as 0.004 mg/m^3 of mustard is reported (69). On-line capability for a gas chromatograph with flame photometric sulfur detection is reported as 0.3 mg/liter, with a direct-reading instrument of ten times this sensitivity projected for the near future (70). The latter is for a very dirty system, namely sulfur dioxide-containing stack gases; a clean system would be easier to devise. It has been possible to analyze for mixed mustard gas, monohydrolysis product and thiodiglycol, using gas chromatography with a flame ionization detector, with as little as 2 μg of sample (71).

The odor threshold described for mustard varies broadly -- 15-120 ppb in water; the variation is probably due to odoriferous impurities in some samples. Dogs and rats can detect down to 0.1 μg per liter of mustard gas in air (72).

The electronic absorption spectrum of mustard gas (73, 74) is not particularly useful for analytical purposes since the maximum occurs at 202 nm (almost in the vacuum ultraviolet) with a molar absorptivity of 4570. The infrared spectrum (75) is useful for identifying mustard or its mixtures.

MAMMALIAN TOXICOLOGY

Human Exposures

Mustard gas can produce severe toxic effects by inhalation, dermal exposure, or oral ingestion. It is very irritating to mucous membranes including structures of the eye and to the skin in quite low concentrations (0.01 to 0.5 mg/cm^2 for the skin) (3, 36). Droplets of liquid mustard as small as 0.0025 mg cause erythema (76).

Concentrations of mustard gas shown to cause eye injury in man (77) are as follows: 200 mg-min/m^3* produces severe to total impairment of vision; 12 to 70 mg-min/m^3 produces mild reddening, but with no incapacitation. However, since 12 mg-min/m^3 produces mild reddening, the no-effect level must be below this. Eight of 13 men exposed at 5 to 10 mg-min/m^3 exhibited signs of eye irritation (78). This compares with a 1.4 mg-min/m^3 per 24 hours that showed no effects in repeated dose animal experiments.

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*Strictly, these are doses. Over a moderately short time range (minutes to hours), the product of concentration and time yields the cited value.

Mustard gas can also cause severe respiratory effects and painful skin burns with blisters in man. From military experience and accidents the estimated median lethal dose is (42):

By inhalation 1,500 mg-min/m^3 = 50 mg/m^3 for 30 min.

By dermal exposure 10,000 mg-min/m^3 = 50 mg/m^3 for 200 min.

Signs of systemic toxicity are generally characterized as radiomimetic since the gastrointestinal signs and bone marrow depression mimic those caused by radiation poisoning (79).

Following the demonstration of mustard gas-induced neoplasia in laboratory animals, retrospective studies in men exposed for certain to mustard during World War I have been made to establish possible carcinogenesis (77, 80). Early studies were equivocal but using British records to the end of 1952, Case and Lea cited by Hassett, 1963 (81) found higher-than-expected death rates, neoplasia of all types, and cancer of the lung and pleura. Increases appear significant, but not dramatic. Wada et al., 1968 (82) traced 500 workers at a mustard gas factory 50 miles from Hiroshima, which had been closed for eight years. Of these, 49 had died of respiratory cancer, 30 histologically confirmed. These workers would have had repeated exposures, as compared to the single or few exposures of men in World War I. The war gas factory case is also complicated by possible exposures to other chemicals manufactured in the facility.

Other data relevant to the evaluation of the carcinogenic risk of mustard gas to man has been summarized in a recent review by the International Agency for Research on Cancer (83).

Within a year or two of its introduction in chemical warfare, note was made that persons exposed repeatedly to small doses of mustard gas became more sensitive to its effects with time. This was recognized as eczematous sensitization by Sulzberger and others in 1945 and 1950 (84, 85). Although the animal work by McNamara (77) failed to demonstrate sensitization in guinea pigs at the exposure levels he used, by analogy with poison ivy extract, sensitization of some individuals could be expected by exposure to concentrations below those which would be primary irritants.

Based on studies with test animals (77), an air concentration limit of 0.003 mg/m^3 for an eight-hour workday was considered safe for workers in clean-up operations. A limit of 0.0001 mg/m^3 was recommended for the general population (77), based on an arbitrary thirty-fold reduction from the level cited above. In a 1973 pollution study by TRW, Inc. for the EPA (42), a level of 3×10^{-6} mg/m^3 has been proposed. Based on extrapolation of air inhalation to water ingestion, a 1.5×10^{-5} mg/l concentration limit has been recommended for water (42).

Experimental Animals

In a 1946 Technical Report, (11), the LCT_{50}'s for mustard gas were measured in various animals. The values are given in Table A-2.

TABLE A-2. LCT_{50} Values for Mustard Gas in Various Animal Species

	LCT_{50} mg min/m^3	Time Range min.
Mouse	860 - 4140	2 - 360
Rat	840 - 1512	2 - 360
Guinea Pig	1700	10
Rabbit	900	10
Cat	700	10
Dog	600	10
Goat	1900	10
Monkey	800	10

LD_{50} values in mice, rats, rabbits, guinea pigs, dogs, and goats by intravenous, subcutaneous, and/or dermal routes are provided by Anslow in a 1946 Report (86, 87). These vary from 0.2 mg/kg intravenously in the dog to 8.6 mg/kg in the mouse, subcutaneously from 2 in the rat to 40 in the goat, and dermally from 20 in the dog to 50 in the goat. Some variation depends upon the vehicle used.

McNamara, 1971 (77) proposed the following limits be placed on air concentrations of mustard for the general population:

0.01 mg/m^3	Maximum
0.00033 mg/m^3	Three-hour exposure
0.00017 mg/m^3	Eight-hour exposure
0.0001 mg/m^3	Indefinite to 72 hours

based upon experiments conducted in dogs, rabbits, guinea pigs, rats, and mice exposed continuously to 0.001 mg/m^3 of purified mustard. Other groups of the same species were exposed to 0.1 mg/m^3 for 6.5 hours, 5 days per week, plus 0.0025 mg/m^3 the remaining time. Exposure times varied from one to 52 weeks. Some rats were held up to 26 additional weeks before autopsy. In the rats 9 of 79 receiving the 0.1 mg/m^3 exposure developed squamous or basal cell carcinomas while none of 79 at the lower exposure level developed similar tumors. These findings were confirmed in a second more complicated experiment where duration of exposure and holding time after exposure together added up to 15 to 20 months.

Exposure as short as three months plus 12 months holding resulted in squamous or basal cell carcinomas. Again, the lower level produced no tumors. No tumors appeared in mice similarly exposed up to 12 months with up to 6 months additional holding. No sensitization in guinea pigs nor teratogenesis, or dominant lethal effects in rats were noted. The dogs showed adverse eye effects after 16 weeks exposure at the higher level, consisting of corneal opacity, pannus, keratitis, vascularization, pigmentation, and granulation.

Mustard in animal model systems has been shown to be carcinogenic (77), and would probably be teratogenic at exposure levels slightly above the higher one used by McNamara. Mustard gas, as well as nitrogen mustard and other alkylating agents, has mutagenic properties. Inactivation of viruses (88), E. coli (89) and T2 bacteriophage (90) has been described.

Other biological and animal data, including metabolism studies, relevant to the evaluation of the carcinogenic risk of mustard gas to man has been summarized in a recent review by the International Agency for Research on Cancer (83).

Very little toxicological information is available on thiodiglycol. Smyth and coworkers, 1941, determined the oral LD_{50}'s of 60 glycols and glycol derivatives in rats and guinea pigs, including thiodiglycol, which is listed as thiodiethylene glycol (91). Using a 10% aqueous dilution he obtained LD_{50}'s of 6.61 (6.10 - 7.16) g/kg for rats and 3.96 (3.44 - 4.56) g/kg for guinea pigs, along with relatively steep dose-response curves. He states that signs of toxicity resemble those of the glycols. The previously cited reference (86) states that thiodiglycol, unlike mustard gas, has no effect on the cardiovascular system after intravenous injections in rabbits or dogs, blood pressure or heart rate is not increased, and vagus nerve irritability is unchanged. In another report (11) thiodiglycol sulfoxide and thiodiglycol sulfone each were fed to groups of 30 mice each at 1000 ppm in the drinking water for 28 days. One of 30 and zero of 30 deaths were recorded, respectively. Mustard gas is said to hydrolyze in water quantitatively to thiodiglycol in a few hours. However, as cited previously (26), one or more short-lived intermediates that can produce toxic effects may be present for 24 hours or so when original mustard:water concentrations are 1:50. As ratios approach 1:1000, the quantities of these intermediates are substantially reduced.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

In view of its low solubility in water and ease of hydrolysis when dissolved, mustard gas cannot travel through the ground in aqueous solution. The volatility is sufficiently high that much of the mustard gas spread on the surface of soil or mixed with earth near the surface is lost to the air by evaporation (3, 76, 92, 93, 94, 95, 96). "On hot days without air motion, concentrations of up to nearly fifty times the required toxic concentration develop [by vapor generation from contaminated soil]" (3).

It is doubtful that mustard gas could be transported through the vascular systems of plants since it would almost surely undergo hydrolysis in the process.

Mustard gas buried deep in the ground where it cannot vaporize or undergo weathering is known to remain undecomposed for many years (29, 97, 98). Even under an immobile layer of water, it persists for long periods (3, 29, 36). Terrain contaminated by high explosive shells with liquid spray or gas clouds will remain vesicant for up to two weeks; the vesicant activity decreases with exposure of the contaminated soil to rain and other environmental conditions (97). Studies by Breazeale and co-workers (93, 94, 95, 96) relate principally to the rates of release of mustard gas by various types of soil under varying conditions, e.g., temperature and humidity. That work has been summarized by Epstein, et al. (29):

"Studies have been made on the vaporization of mustard [H] at 70° to 78°F and 27% to 35% relative humidity after experimental application to calcareous soils and on the effect of added moisture to several types of soil under controlled conditions. Recoveries from the calcareous soils varied from 7% to 32% when sampled for about 6 hours; when sampling was continued until no more H vaporized (15 to 55 hours), the percentages of the initial contamination recovered increased, varying from 12% to 66%. Both the rate of vapor generation and the percent of mustard recovered in the vapor state depended on the pH, moisture content, and physical constituents of the soils. Finer soils gave a lower return of vapor than did coarse, sandy soils. Considering the effects of particulate size in a soil irrespective of the chemical components, particulates above 1 mm in diameter would play very little part in the adsorption, and thus in the retention of an agent such as H. Below 1-mm-diameter sizes, as the particle size decreases, the surface area greatly increases, thereby increasing the adsorbing power of the soil. Plots of vapor return versus moisture content go through a maximum which varies with the type of soil. The state of the unrecovered agent remaining in the soil was not determined." Thus, some of the mustard may be tied up chemically by the soil, as implied by Deuel, Huber and Iberg (99), who claimed the formation of silicate esters by reaction of montmorillonite clay in which sodium had replaced exchangeable hydrogen:

$$\text{Clay} \text{———} SiO^{-}Na^{+} + ClCH_2CH_2\text{-}S\text{-}CH_2CH_2Cl + H_2O$$

$$\longrightarrow \text{Clay} \text{——} SiO\text{-}CH_2CH_2\text{-}S\text{-}CH_2CH_2OH + NaCl + HCl$$

Their observations were subsequently disputed by Brown, Greene-Kelly and Norrish (100). Such clay materials as bentonite, attapulgite and vermiculite proved unsuccessful as barriers to mustard vapor (44). How far mustard gas can permeate porous soil is an important, unresolved question; the answer could influence the ways in which sampling, analysis and disposal of the agent are accomplished.

Most probably, any mustard gas that may exist at this time should be present only as pockets of liquid, perhaps dissolved in discarded oil, or absorbed on an inert anhydrous soil medium. Any mustard residues that may be found in pockets of soil or trapped in structures or containers should be removed and destroyed using OSHA recommended protective gear and procedures.

Insects:

Mutagenicity has been demonstrated in the fruit fly Drosophila (101). A bean beetle insecticide of 0.01% mustard gas in ether has been used (102).

Microorganisms:

The response to sulfur mustard has been studied extensively in microorganisms. Principally, these investigations utilized mustard along with other alkylating agents to ascertain the molecular mechanisms of action on cells. The objective was to determine the mechanism of toxic action to the whole animal.

Herriott in 1948 (88) compared a number of bacteria, phages, viruses and enzymes for their sensitivity to 1×10^{-3} M mustard gas and found that animal and bacterial viruses are generally inactivated at rates similar to yeasts and bacteria. All of the microorganisms were more sensitive than the most sensitive enzymes tested. He also noted that various strains of the same bacteria differed in sensitivity to contact with mustard.

Early studies of microbial reactivity to mustard compounds were performed with the molds Neurospora sp or Aspergillus sp. Hockenhull (103) examined Aspergillus nidulans mutations after exposure to mustard

gas. He studied cystine-dependent mutants derived from 640 viable exposed conidia spores and found 68 different morphologues. Only 2 morphologues were noted in 1480 viable unexposed spores. Stevens and Mylrotie 1952, (104) utilized nutritional dependent mutants of Neurospora crassa to determine if mustard compounds would induce mutational reversion to nutritional independence. Various nutritional mutants had different rates of reversion to similar mustard dosage.

Stevens and Mylrotie, 1953, (105) also studied mustard mutagenicity with Neurospora crassa. They were able to produce a number of nutritional mutants requiring amino acids, vitamins or other growth factors. The mutants were induced by mustard concentrations of between 6.0×10^{-4} and 5.0×10^{-3}M. They determined that various mutations could be induced to revert to wild type phenotypic response by a second treatment with mustard or ultraviolet irradiation. Paraaminobenzoic acid and leucine reversions were principally by suppressor mutation or back mutation. Leucine reversions were principally by back mutation.

More recent studies have dealt with molecular mechanisms of attack on viable bacterial cells and viruses. In part this was because of their simple structure and because the ease of culturing made their utilization desirable.

Papirmeister, 1961, (90) studied the mechanism of T_2 bacteriophage inactivation in great detail. He observed that mustard at low dosage inactivates the phage when existing free or preadsorbed to susceptable cells. The principal effect was upon the DNA of the phage rather than protein moities. Mustard sterilization of the host bacterial cells allowed normal T_2 phage to reporduce and provide normal replication of viral DNA. It was observed that mustard-inactivated particles could be reactivated in host bacteria by multiplicity reactivation, demonstrating that a viable genome can be reconstituted from its undamaged portions.

Papirmeister and Davison, 1965, (89) also investigated the lethality of sulfur mustard for nutritional mutants of E. coli 15 requiring thymine, arginine and uracil. Mustard at 1.5 to 2.0×10^{-4} M inhibited DNA synthesis. However, overnight incubation in a medium containing thymine, arginine and uracil provided some recovery, as noted by the production of normal-appearing colonies. This suggests that cell damage may be reversible. They postulated that sulfur mustard causes interstrand crosslinks in DNA moieties.

These observations of cell repair after mustard treatment spurred further research into possible mechanisms. Lawley and Brooks, 1965, (106) observed that the initial effect was at guanine base moieties. Utilizing three E. coli strains, they observed that 0.31 mM mustard alkylated the DNA's equally. They theorized that principal toxicity was prevention of total DNA replication because of interstrand crosslinkage of difunctionally

alkylated guanine. They observed that certain of the bacterial strains were capable of excising the crosslinks. Further support of the cross linkage mechanism was provided by Kohn et al., 1965, (107) but using nitrogen mustard. Papirmeister and Davison, 1965, (89) concluded that E. coli 15 (thymine, arginine and uracil-requiring) depolymerized a significant portion of its DNA after sulfur mustard treatment. A higher rate of mustard-bound DNA degradation than loss of normal DNA was noted. Resumption of DNA replication was initiated as the excision of DNA alkylation products occurred. No difference in the excision of mono- and bifunctionally alkylated guanine base moieties was observed. Venitt, 1968, (108) observed that E. coli B/r selectively excised di(guanin-7-yl) ethyl sulfide from its DNA after exposure to 6 mg/ml mustard gas. A mustard-sensitive strain B_{s-1} did not excise the crosslinked DNA moieties. The mean lethal dose for E. coli B/2 was 6.0 mg/ml mustard and 0.8 mg/ml for the B_{s-1} strain.

Papirmeister et al., 1968, (109) reviewed the protection and reversal of lethal mustard damage in bacterial cells and virus. They stated that the most pronounced lethal action occurred with utilization of proliferating entities. Non-dividing or non-replicating organisms and viruses were less sensitive. DNA was the most sensitive site and the mechanism with difunctional mustards was the selective formation of guanine interstrand crosslinks. The excision of crosslinks was necessary for survival. This crosslinkage required much more monofunctional mustard than bifunctional mustard. In addition to DNA crosslinkage, other mustard mechanisms are 1) breakage of phosphodiester backbone of DNA, thereby sensitizing the DNA to action of exonuclease, 2) increased hydration of the DNA around charged quaternary nitrogen atoms during alkylation, thus interfering with phage DNA injection in tailed species, 3) interference by crosslinks with quaternary structure or packaging of DNA with basic proteins during phage maturation.

Plants

Phytotoxicity: An evaluation of 300 species of plants by Fichet (110) indicated plant leaf tissue was susceptible to liquid mustard gas droplets. Circular patterns of dead tissue developed where mustard droplets touched the leaf. These spots expanded to about double the drop size as the liquid mustard gas dispersed through leaf vessels. Small doses did not kill plants (110). Treatment with mustard gas vapor for two hours caused injury to young shoots on several potted flower plants (111). The leaves wilted, shriveled and fell. However, new leaves came out on the plants and the plants recovered. Liquid mustard gas can cause destruction of bean root cells in 10 minutes, and mustard gas may inhibit seed germination (barley and wheat) by destroying the embryo (112). Most evidence points to plasmolysis or protoplasmic contraction as a cause of leaf death when treated with mustard gas (111, 113).

Mustard gas vapor on pollen and egg cells can result in mutations found in the new generation (103, 114) or, as in most cases, renders the plant sterile, unable to produce seed (114). The sterility found in corn after treatment with mustard gas is directly correlated with length of time and mustard concentration during treatment (114). Corn pollen exposed to saturated mustard gas vapor for longer than two minutes produces essentially barren ears (114).

Treatment of dormant barley and wheat seeds with mustard has been shown to decrease germination of seed and alter fertility and mutation rate in plants grown from treated seed (115).

A biological screen for herbicidal activity of thiodiglycol at 0.1 and 1 pound per acre has been conducted at Ft. Detrick (116). There was no effect of thiodiglycol on any of the plants tested (aerial application to beans, oats, rice, soybeans, radishes and morning glories).

EXISTING STANDARDS

No legally mandated or industrially accepted standards have been established for mustard gas.

However, Maximum Permissible Concentrations (MPC's)* of certain chemical agents in water were officially stated in SOLOG agreement 125 by the quadripartite nations (U.S., U.K., Canada and Australia). The MPC for sulfur mustard was set at 2.0 mg/l (117).

* MPC's determine whether or not contaminated, raw water must be subjected to decontamination. They are also used to check the finished water to be sure that the decontamination procedure has been successful and that the final product water is fit to drink.

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APPENDIX B

LEWISITE/LEWISITE OXIDE

Lewisite is quickly converted to lewisite oxide on exposure to environmental moisture. For this reason, the two compounds are considered together. Lewisite would only be found intact in the ground or in structures if rigorously protected from moisture. Nevertheless, lewisite is important for the present discussion because the toxicities of lewisite and lewisite oxide are probably similar, and the literature deals chiefly with the former; thus toxicity values for lewisite may be considered as applying to lewisite oxide.

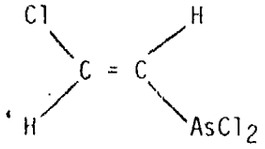
ALTERNATIVE NAMES

LEWISITE: Lewisite (Chem. Abstr. through 1961); dichloro(2-chlorovinyl) arsine; arsine, dichloro(2-chlorovinyl)-(Chem. Abstr. 1962-1971); arsonous dichloride, (2-chloroethenyl)-(Chem. Abstr. after 1971).

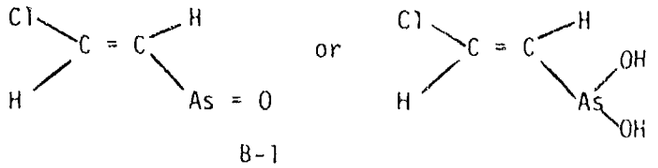
LEWISITE OXIDE: 2-Chlorovinylarsonous acid; arsine, (2-chlorovinyl) oxo (Chem. Abstr. 1962-1966); arsonous acid, (2-chlorovinyl); arsonous acid, (2-chloroethenyl); 2-chloroethenearsonous acid; arsine, (2-chloroethenyl) oxo; ethenearsonous acid, 2-chloro (Chem. Abstr. 1947-1961 and [illegible] at least through 1971); ethene, 1-arsenoso-2-chloro; ethylene, 1-[illegible] (Chem. Abstr. 1937-1956); ethylenearsonous acid, 2-chloro.

PHYSICAL AND CHEMICAL PROPERTIES

LEWISITE:
CAS Reg. No. 541-25-3, 50361-05-2
Defense Department Symbol: L
Toxic Substances List: CH29750
Wiswesser Line Notation: G-AS-G1U1G
Molecular formula: $C_2H_2AsCl_3$
Structural formula:



LEWISITE OXIDE (in equilibrium with the corresponding dibasic acid):
Possible Defense Department Symbol: LO
(Not in Toxic Substances List)
Wiswesser Line Notations: O-AS-1U1G or Q-AS-Q1U1G
Molecular formulas: C_2H_2AsClO or $C_2H_4AsClO_2$
Structural formulas:



Lewisite, although known in an impure state since 1904, was first characterized by Professor W. Lee Lewis of Northwestern University in 1918, too late to be employed as a vesicant agent in World War I (1). It was manufactured during World War II, stored in 1-ton containers, but evidently not loaded into munitions.

Lewisite is formed by the Lewis acid-catalyzed addition of arsenic trichloride to acetylene (2, 3). Catalysts for this reaction are aluminum chloride (3, 4), cuprous chloride (3, 4, 5) and mercuric chloride (3, 4, 6).

$$AsCl_3 + HC{\equiv}CH \xrightarrow{\text{catalyst}} Cl_2AsCH{=}CHCl$$

Plant run lewisite is a complex mixture containing the cis and trans isomers of lewisite, bis-(2-chlorovinyl)chloroarsine, tris-(2-chlorovinyl)-arsine, and arsenic trichloride. Mercuric chloride catalyst and metallic mercury derived from the mercuric chloride may also be present if that catalyst was employed.

Efforts were made to minimize the non-lewisite components by proper control of the manufacturing process. Of these components only bis-(2-chlorovinyl)arsine has a toxicity comparable to that of lewisite, and it is less volatile. The content of cis-lewisite was generally in the order of 10% (7), and there seemed to be no need for eliminating this component, especially since the two isomers are of similar toxicity (8).

Elucidation of the cis- and trans- structures required isolation of these isomers (7). They could not be completely separated by fractional distillation since a good vacuum was needed to prevent decomposition. At low pressures, the difference in their boiling points (which is only 26.8°C at atmospheric pressure (9)) was too small for efficient fractionation, e.g., only about 7-8° at 7 mm (7). The separation of the isomers was therefore completed through their hypochlorous acid or hydrogen peroxide oxidation to the corresponding chlorovinylarsonic acids. The acids were then purified by recrystallization and reduced with sulfur dioxide in concentrated hydrochloric acid to form the respective lewisite isomers (7) once again. Vapor pressures of the isomers have been measured accurately over a large range (9, 10, 11). The dipole moment was used to establish the structure (12), which was also confirmed by electron diffraction studies (13). trans-Lewisite is said to be converted to the cis-isomer by ultraviolet light in the course of thermal decomposition (3), or catalytically (8).

The data in Table B-2 pertains to lewisite (cis-trans mixture, presumably) and two related organic impurities. Infrared spectra of lewisite and the impurities were determined by Thomas (14).

TABLE B-1. Physicochemical Constants of cis- and trans-2-Chlorovinyldichloroarsine (9).

Property	cis-Isomer.	trans-Isomer.
Freezing point	-44.7^{o}	-1.2^{o}
Vapor pressure (mm. Hg) at 25^{o}	1.562	0.40
log p (mm. Hg)	8.4131 - 2450.2/T	48.660 - 13.297 log T -4815.3/T
b.p./760 mm.	169.8^{o}	196.6^{o}
Latent heat of vaporization at 25^{o}, $L_{v}^{25^{o}}$ (cal./g.-mol.)	11,220	15,150
L_{v} at the b.p.	11,220	9,620
Molar b.p. depression	34.7^{o}	45.6^{o}
$d_{4}^{25^{o}}$	1.8598	1.8793
$d_{4}^{t^{o}}$	1.9018 - 0.00168t	1.9210 - 0.00167t
$n_{\underline{D}}^{25^{o}}$	1.5859	1.6076
$n_{\underline{D}}^{t^{o}}$	1.6002 - 0.000575t	1.6201 - 0.0050t
$[R_{\underline{L}}]_{\underline{D}}$, molar refraction in ml/mole	37.388	38.089
n at 25^{o} (g./cm/-sec.)	0.0169	0.0205
log n	590/T - 3.751	699/T - 4.033
Dipole moment (12) in e.s.u. x 10^{18}	2.61	2.21

TABLE B-2. Ultraviolet Absorption Parameters of Lewisite and Related Impurities (15).

	Absorption maximum (nm)	Molar absorptivity
Lewisite	214	10,000
bis-(2-Chlorovinyl)chloroarsine	209	14,000
tris-(2-Chlorovinyl)arsine	207	26,000

Lewisite is said to have a solubility of 0.5 grams per liter (16), but this is virtually meaningless in view of its very high rate of hydrolysis. The hydrolysis of lewisite is complex, involving several reversible reactions (17):

$$ClCH{:}CH{\cdot}AsCl_2 + 2H_2O \underset{}{\overset{\text{fast}}{\rightleftharpoons}} Cl{\cdot}CH{:}CH{\cdot}As(OH)_2 + 2HCl$$

$$Cl{\cdot}CH{:}CH{\cdot}As(OH)_2 \underset{\text{slow}}{\rightleftharpoons} H_2O + Cl{\cdot}CH{:}CH{\cdot}AsO \underset{\text{slow}}{\rightleftharpoons} (Cl{\cdot}CH{:}CH{\cdot}AsO)_x$$

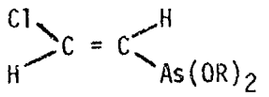
(geminal diol) (oxide) (polymer)

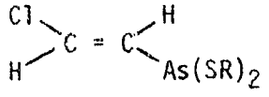
The first equilibrium (17, 18) lies on the side of the lewisite formation above a hydrochloric acid normality of 2. There are actually three hydrolysis products in true equilibrium with one another; the weakly acidic water-soluble geminal diol, the benzene-soluble oxide, and the relatively insoluble polymer. Lewisite oxide is about 1% soluble in water (17), over 2% soluble in seawater (19), and somewhat more soluble in slightly alkaline solution (17). The trans-oxide* melts at 82.5-84.0°, its polymer at 140° (20). The cis-oxide* melts at 107.5-108.5° and appears to be stable and not to polymerize (20). It is to be noted (21) that lewisite hydrolyzes more rapidly than bis-(2-chlorovinyl)arsine.

At higher pH levels, trans-lewisite oxide is cleaved by hydroxyl ion to give acetylene and inorganic arsenite; this occurs even in the cold (7, 17, 22). Above pH 10, the reaction should be complete within a day (17). The cis-compound must be heated to 40° to react with sodium hydroxide solution, then giving vinyl chloride (along with acetylene, it would seem) and inorganic arsenite (7, 8, 22).

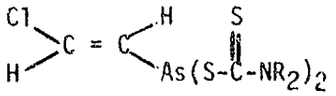
*Nomenclature in reference (20) was confused; Bartlett's "isomer I" must have been the trans-form, since it liberated acetylene readily in the cold (8).

Lewisite reacts with oxygen and sulfur nucleophiles to form derivatives (17) of the types





and



Cyclic dithioethers are especially stable (17, 23). Lewisite also forms reversible adducts with dioxane and thioxane.

Heating causes lewisite to disproportionate to arsenic trichloride, tris-(2-chlorovinyl)arsine, and bis-(2-chlorovinyl)chloroarsine (22). Chlorine reacts with anhydrous lewisite to cleave the carbon-arsenic bond, yielding arsenic trichloride and dichloroethylene (18). Lewisite (or the oxide) is easily oxidized to 2-chlorovinylarsonic acid in aqueous solution by a variety of oxidants (7, 18), including hypochlorous acid, hydrogen peroxide, chloramines and iodine. It is also said to undergo oxidation gradually in fresh water (24) or seawater (19). The conclusion has been drawn (25) that it should behave in a manner analogous to sodium arsenite (26), which is oxidized in the soil, presumably by micro-organisms. The oxidized product, 2-chlorovinylarsonic acid, is said to have markedly decreased toxicity (27) or to produce no physiological effects (3).

Lewisite applied to soil quickly volatilizes or is converted to the still-toxic lewisite oxide may be chemical or microbiological (25, 28, 29), which cannot, however, so easily reach the target organism, man.

Lewisite at 30 ppm (i.e., the oxide) is 98% removed from drinking water with 600 ppm of activated charcoal, followed by coagulation and filtration (30).

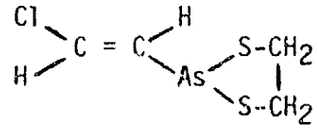
Lewisite and its "oxide" interfere with the pyruvate oxidase system (3, 31) probably by reaction with dihydrolipoic acid (18). The voluminous literature on this subject and on British Anti-Lewisite (BAL, 2,3-dimercapto-1-propanol) (32, 33) will not be reviewed here.

ANALYTICAL METHODS

Lewisite in water (i.e., the oxide) has been detected by conversion to arsine with zinc and application of some form of the Gutzeit test (34, 35, 36, 37). This is the commonly used approach for military water testing, with detectibility down to 4 mg/liter, which is the required level met by the XM 256 sampler (35). (Note: See methods for detecting arsenic.) A second principle of detection entails treatment with base to liberate acetylene, which is trapped with cuprous chloride to form a reddish derivative (38). Lewisite and lewisite oxide react with an

aqueous solution containing cupric ion and piperidine to give a brilliant red color (39). A molybdenum blue test is sometimes employed for detecting lewisite (36). Organic reagents for lewisite include the following: γ-(4-nitrobenzyl)pyridine (40); di-p-biphenylthiocarbazone (41), which is not sufficiently specific (36); ergosterol on silicia gel (42), sensitive to 10 micrograms; the rather specific m-dinitrobenzoyleneurea (43); the fairly sensitive sodium p,p'-dinitrostilbene-o-o'-disulfonate (43) that can be used to detect 30 micrograms of lewisite (43); and a number of other polynitro compounds (43). According to Northrop (44), dogs and rats can be trained to detect lewisite by its odor. Lewisite is said to have an odor threshold in water (to humans) of 100-300 ppb (45). The mass spectra of lewisite and bis-(2-chlorovinyl)chloroarsine have been determined (46).

Little attention has been paid in recent years to laboratory procedures for the quantitative analysis of lewisite and lewisite oxide. Thus, the derivatization of lewisite oxide to permit extraction and gas chromatography has been successfully attempted in only one known instance (23):

aqueous lewisite oxide + ethanedithiol → 

Based on the analogy of the behavior of lewisite to that of arsenious oxide (47), other approaches to gas chromatography are possible. For example, lewisite oxide should be extractable into toluene as the diiodide after the latter is formed with potassium iodide in 10N sulfuric acid. It should be possible to chromatograph the diiodide. Other derivatives of inorganic arsenic have also been formed to permit analysis by gas chromatography (48), and these may be looked upon as models for lewisite oxide derivatives of analytical utility.

MAMMALIAN TOXICOLOGY

Human Exposures

Lewisite is a skin-damaging warfare agent that acts not only as a contact poison, but also as an inhalation and eye poison. The skin-damaging effect takes place immediately. Erythemata form on the surface of the skin with doses of about 0.05 to 0.1 mg per square centimeter of skin surface (16). Concentrations of 0.2 mg per square centimeter positively lead to blister formation. Blisters on the surface of the skin are caused by gaseous lewisite after about 15 minutes dermal exposure to concentrations of 10 mg/l. Inhalation of concentrations of 0.05 mg/l for 30 minutes or 0.5 mg/l for 5 minutes is considered lethal. An inhalation exposure of 0.05 mg/l for 15 minutes produces severe intoxication which causes an incapacity for several weeks. A lower concentration of 0.01 mg/l causes inflammation of the eyes and swelling of the lid after 15 minutes. British Anti-Lewisite (BAL) is a specific antidote for lewisite contact and systemic poisoning (32, 33).

Inhalation of 48 mg/m^3 of lewisite for 30 minutes is fatal for man (Prentiss, 1937 (49)). Skin absorption of 1.4 ml of lewisite (20 mg/kg) by man results in death in 3 hours to 5 days (Sollman, 1957 (50)). The toxicity of lewisite for man is summarized in Table B-3.

TABLE B-3. Toxicity of Lewisite for Man (51).

	Vapor approx LC_{50} mg min/l	Liquid dose mg
Death (by inhalation)	1.2-1.5 (est)	
Death (by body exposure)	100 (est)	2,800 (est)
Vesication of skin (bare)	1.2-1.5 (est)	0.014
Serious corneal damage	1.5 (est)	0.1 (est)

The physiological and toxicological properties of both lewisite vapor and lewisite liquid as they affect the eyes, respiratory tract, skin, and systemic systems are detailed in the review paper of Gates et al., 1946 (51), which summarizes all the published work up to that date. There are no publications in the literature on the animal toxicology of lewisite after 1946.

The toxicity of lewisite oxide to humans does not appear to have been studied as such. However, the oxide is itself necrosant, and it is assumed that the arsenical residue passes into the circulation, fixes itself in various organs, and sets up a general systemic poisoning, typical of arsenical compounds.

A maximum permissible concentration was established by the Army Surgeon General for inorganic arsenic, i.e., 2 mg/liter as arsenic (52), and this has been applied also to lewisite and any other arsenicals, i.e. 2 mg/liter as arsenic (53). This is a seven-day emergency drinking water standard (54). Note that the Army Surgeon General established a tolerance level for lewisite (as arsenic), for consumption between one week and one year, of 0.2 mg/l (55).

Experimental Animals

The toxicity of lewisite to experimental animals, both by skin application (dermal LD_{50}'s) and vapor exposure (LC_{50}) has been extensively investigated. The results of all this work up to 1946 are very adequately summarized by Gates et al., 1946 (51). Additional data from the Toxic Substances List, 1974 (56) are:

Oral LD_{50} rat: 50 mg/kg

Inhalation LC_{100} mouse: 150 mg/m^3

Unpublished experiments by McCreesh and Koviak (57) indicate that the LD_{50} in the mouse is between about 5 and 15 mg/kg depending on the concentration (1.6% in seawater, 5.8% in PEG200). Inflammation occurred in eyes of rabbits with application of 0.10 ml of 1.0 mg/cc solution and permanent damage was caused by 0.10 ml of 10 mm^3/cc.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

It may be assumed that any lewisite that is exposed to moisture, even that in the soil of a relatively dry region, soon converts to lewisite oxide. Nothing is known of the long-term stability of lewisite oxide in the soil environment. As mentioned under "PHYSICAL AND CHEMICAL PROPERTIES," the possibility exists for oxidation of lewisite oxide in soil to 2-chlorovinylarsonic acid. The conversion to inorganic arsenic takes place in alkaline solution at measurable rates. Possibly this can also take place slowly in the soil environment at lower effective pH levels. Since the amount of lewisite lost from soil by evaporation was far less than that applied experimentally, as compared to mustard (29), one may conclude that lewisite oxide (formed by lewisite hydrolysis) is much more persistent in soil than mustard.

Animals

Mammals: No information retrieved.

Birds: No information retrieved.

Fish: Price and von Limbach (58) report the following toxic doses for lewisite in water: golden shiners, 0.2 ppm; bluegills, 0.5 ppm; bass, < 2.0 ppm. Lewisite seems to lose toxicity to fish after 50 days in water. Bauer et al. (59) observed sunfish for 24 hours. None died in 6.5 and 3.25 ppm dilutions, but they showed signs of stress.

Tadpoles: Price and Limbach (58) also studied tadpoles. Toxicity was noted at 0.5 ppm. Survival was prolonged with greater population densities than 1 or 2 animals per aquarium, i.e., 16-32 per tank.

Invertebrates: No information retrieved.

Microorganisms: No information retrieved.

Plants

Phytotoxicity: Lewisite is apparently phytotoxic. Immersion of bean roots in liquid lewisite for 2 seconds caused destruction of the living cells (60). Lewisite vapor is implicated in the death of vegetation in lewisite shell target areas (29).

Food Chain

Because of its extreme phytotoxicity there would seem to be little chance for the bioconcentration of lewisite oxide through the food chain. Animals feeding on other animals killed by or otherwise containing lewisite oxide could pick up the arsenic, which concentrates in the internal organs (61). The problem thus becomes that of transport and dispersal of arsenic through the ecosystem, rather than lewisite oxide, as such.

EXISTING STANDARDS

There are no standards for lewisite or lewisite oxide.

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APPENDIX C

METHYLPHOSPHONIC ACID

ALTERNATIVE NAMES

Methylphosphonic acid: phosphonic acid, methyl-(Chem. Abstr. 1947 ff); methanephosphonic acid (before 1947).

PHYSICAL AND CHEMICAL PROPERTIES

CAS Reg. No.: 993-13-5.
Toxic Substances List: Not listed.
Edgewood Arsenal Number: CS 833,534.
Wiswesser Line Notation: QPQO&1.
Molecular formula: CH_5O_3P.
Structural formula: $(CH_3)P(=O)(OH)_2$.

Melting Points: Pure, 101-104°C (1), 104-106°C (2), 107-107.5°C (3); sodium salt, 435-440°C (1); aniline salt, 149-150°C (1, 4).

Dissociation Constants: $pK_1 = 2.38$ (2); $pK_2 = 7.74$ (2).

Infrared Absorptions: 1312, 1149 and 889 cm^{-1} (5).

Solubility in water: Very soluble; calcium salt is also soluble (6).

Preparation: Methylphosphonic acid (MPA) is prepared by isomerizing trimethyl phosphite to dimethyl methylphosphonate and hydrolyzing this ester (2, 7) with acid or by hydrolyzing isopropyl methylphosphonate (8) or methyl methylphosphonate (1) with acid. Another pathway involves formation of the anhydride, $(CH_3PO_2)_2$, either by heating methyl methylphosphonochloridate at 150-160°C/1-2 mm pressure, which evolves chloromethane (3), or by partial hydrolysis of methylphosphonodichloridate (9). The anhydride is hydrolyzed to MPA (3, 10). MPA may also be prepared by hydrolysis of $(CH_3)PI_4$ (4). Although it has been implied that MPA is a hydrolysis product of the nerve agent GB under environmental conditions (11), GB requires heat and strong acid to effect hydrolysis past the isopropyl methylphosphonate stage. MPA is one of the compounds identified in the products resulting from pyrolysis of sodium fluoride and sodium isopropyl methylphosphonate at 425°C (12).

Stability: Methylphosphonic acid is quite stable. Boiling with concentrated HNO_3 or H_2SO_4 yields inorganic phosphate (6). Oxidation can also be effected with potassium permanganate in 15N nitric acid (13). Anodic oxidation of MPA to inorganic phosphate has been reported; 100 ml of a solution containing 0.722g MPA and 11 ml of 45% NaOH was

electrolyzed between two platinum mesh electrodes at 9 amperes for 1 hours to give 100% conversion of MPA to phosphate when a current density of 0.4 amp/cm² was used (14). MPA was oxidized to phosphate by ozone alone (15) or better in the presence of a cobalt salt (16). A small fraction (1.5%) of GB (isopropyl methylphosphonofluoridate) was converted to MPA on 9-hour incubation with rat serum (17). Methylphosphonic acid (^{32}P), administered intraperitoneally, was not degraded to phosphate in the rat, and 9% of the MPA was excreted in the urine in 48 hours (18). "With the exception of the 2-chloroethylphosphonates which readily cleave at the P-C bond the evidence ... indicates that the P-C bond of methyl-, ethyl-, and phenylphosphonates resists catabolism by higher animals or plants." (19). One exception to this appears to be a minor amount of P-phenyl bond cleavage of the fungicide Inezin by rice plants (19, 20). "Among microorganisms [however] ... the capacity to catabolize the P-C bond appears to be widespread" (19). In particular, this has been demonstrated with methylphosphonic acid for E. coli, indirectly by Zeleznick, Myers and Titchener (19, 21, 22), and directly by James, Myers and Titchener (23). It would appear that carbon-phosphorus cleavage by E. coli may be inferred for compounds such as phenylphosphonic acid, 2-aminoethylphosphonic acid and chloromethylphosphonic acid (24). In all probability, of these substrates, only 2-aminoethylphosphonic acid is degraded by a mechanism utilizing the enzyme "phosphonatase", which was isolated from B. cereus (25). Strains of Pseudomonas aeruginosa produced two red phenazine pigments (aeruginosin A and B) in addition to pyocyanine, when incubated with MPA as the sole phosphorus source, according to Neuzil et al. (26).

ANALYTICAL METHODS

Water samples containing MPA were acidified and freeze-dried to remove the water. The residue remaining was treated with bistrimethylsilyl acetamide to form the trimethylsilyl ester of MPA. Attempted analysis by GLC on a 6' x 1/4" 3% Dexsil-300 column at 120°C (12) failed. Analysis of MPA in the presence of other alkylphosphonic acids by paper chromatography and paper electrophoresis has also been described (27). MPA can be titrated as a monobasic acid in acetonitrile, ethyl acetate or pyridine (28). Values of pK_a for MPA in various alcohols have been determined (29).

MAMMALIAN TOXICOLOGY

No information or data is available for either humans or experimental animals.

ENVIRONMENTAL CONSIDERATIONS

No information is available on behavior in soil and water, on exposure of natural animal populations, or on food chain transferral of methylphosphonic acid.

Plants

Some forms of phosphonic acids are known to have hormone-like properties in plants and can thus affect plant growth and development (30, 31). These effects are caused by the chlorphenoxymethane phosphonates (30) which appear to mimic auxin activity in plants (probably through similarity of the molecule or its breakdown product to 2,4-D) and by 2-chloroethylphosphonic acid, which is known to release ethylene in plant tissue (31). Hambrock et al. (32) demonstrated that wheat plants could convert O-pinacolyl methylphosphonofluoridate to methylphosphonic acid; however, they report no ill effects of methylphosphonic acid on the wheat plants (32). Methylphosphonic acid has been aerially sprayed at 0.1, 1.0, and 10.0 pounds per acre on Black Valentine beans, soybeans, morning glories, radishes, oats and rice (6, 33). Test results differ between two trials (6, 33), with one test trial indicating a slight effect of methylphosphonic acid on soybean and morning glory at 1.0 pound per acre (highest rate tested) and the other test trial indicating a slight stunting effect on oats at 0.1 pound per acre. At 1.0 pound per acre, there were formative effects on Black Valentine beans and soybeans, chlorosis and necrosis on morning glories, and slight stunting in oats. At 10 pounds per acre, there was severe contact injury and stunting of Black Valentine beans, soybeans, morning glories and rice, and severe contact injury on radishes and oats. In another study, eight aquatic plant species were tested for their response to MPA. The table below summarizes the data (11).

TABLE C-1. PLANT RESPONSES* AT DIFFERENT CONCENTRATIONS OF METHYLPHOSPHONIC ACID

Organism	Concentration (ppm) 1000	100	10	1	0.1
FLOWERING PLANTS					
Lemna minor	D	X	0	0	0
Lemna perpusilla	D	0	0	0	0
Lemna valdiviana	D	X	0	0	0
Spirodela biperforata	D	X	0	0	0
Spirodela oligorhiza	D	X	0	0	0
Wolffia papulifera	D	0	0	0	0
ALGAE					
Ourococcus bicaudatus	D	D	X	0	0
Chlorella pyrenoidosa	D	D	X	0	0

*D = Death; X = Decrease in growth rate; 0 = no effect

Propylphosphonic acid has been demonstrated to retard growth in bean plants growing in nutrient solution containing 1 mM propylphosphonic acid (34). Height, fresh weight of tops and dry weight of roots were reduced about 50 percent as compared with control plants growing in nutrient culture not containing propylphosphonic acid (34).

EXISTING STANDARDS

No information available.

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APPENDIX D

ISOPROPYL METHYLPHOSPHONATE

ALTERNATIVE NAMES

Isopropyl methylphosphonate; phosphonic acid, methyl-, monoisopropyl ester (Chem. Abstr. 1967-1971); phosphonic acid, methyl-, mono (1-methylethyl) ester (Chem. Abstr. after 1971; CAS Registry Handbook 1965-1971); phosphonic acid, methyl-, isopropyl ester (Chem. Abstr. 1947-1966); methanephosphonic acid, isopropyl ester; isopropyl hydrogen methylphosphonate; O-isopropyl methylphosphonic acid; 2-propyl methylphosphonate.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Reg. No. 1832-54-8
Toxic Substances List: Not listed
Wiswesser Line Notation: QP0&1&0Y
Molecular Formula: $C_4H_{11}O_3P$
Structural formula: $((CH_3)_2CHO)\ (CH_3)P(=O)OH$

Isopropyl methylphosphonate (IMP) is a liquid boiling at 97-98°C/0.08 torr (1) and 123-125°/0.2 torr (2). It has a refractive index of n_D^{20}=1.4228 and a specific gravity of 1.1091 at 20°C (1). By analogy with ethyl methylphosphonate, the pK_a must be about 2.0 (2). IMP is difficult to extract from aqueous acid with organic solvents; thus, the partition coefficient (K=conc. in organic phase/conc. in aqueous phase) for methylene chloride/acidified water was reported as 0.0024 (3). The rate of hydrolysis of IMP to methylphosphonic acid at ambient temperature has not been reported. However, phosphate monoesters are generally very stable near neutrality, hydrolyzed only very slowly in alkaline solution and rapidly hydrolyzed in hot strong acid (2). The hydrolysis of IMP must be immeasurably slow at neutral pH and 130.5°; in 1N benzenesulfonic acid the rate constant was 0.040 hr^{-1} at 91.3°C and 0.175 hr^{-1} at 102.7°C (2). This would indicate a half-life of about 1900 years at pH 1, and much more at neutral pH. Isopropyl methylphosphonic acid may be prepared by the partial hydrolysis of diisopropyl methylphosphonate by barium hydroxide (1), or by hydrolysis of isopropyl methylphosphonochloridate with cold aqueous acetone (2).

Isopropyl methylphosphonic acid is not subject to enzymatic degradation in the rat. When IMP was administered intraperitoneally, 40% was excreted in the urine after 48 hours (4). In 72 hours, 85% was excreted in rat urine (5).

ANALYTICAL METHODS

Aqueous solutions of IMP can be analyzed by gas chromatography using FID/GC on a 6' x ¼" Tenax column at 200°C. Concentrations of 1000 ppm can easily be detected, but the method is not quantitative (3). IMP may be determined quantitatively by acidifying the aqueous solution, freeze-drying,

and treating the residue with bistrimethylsilylacetamide to form the trimethylsilyl ester, which may be analyzed by FID/GC using a 6' x ¼" 3% Dexsil 300 column at 120°C (3). Aqueous solutions of IMP may be diluted 10:1 with 2-propanol, then treated with ethereal 1-diazapropane to give propyl isopropyl methylphosphonate; this can be determined by gas chromatography (6).

MAMMALIAN TOXICOLOGY

No information available.

ECOLOGICAL CONSIDERATIONS

No information available.

EXISTING STANDARDS

No information available.

LITERATURE CITED

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APPENDIX E

DIISOPROPYL METHYLPHOSPHONATE

ALTERNATIVE NAMES

Diisopropyl methylphosphonate; DIMP; phosphonic acid, methyl-, bis-(1-methylethyl) ester (Chem. Abstr. after 1971); phosphonic acid, methyl-, diispropyl ester (1947-1971); methanephosphonic acid, diisopropyl ester

PHYSICAL AND CHEMICAL PROPERTIES

CAS Reg. No. 1445-75-6
Toxic Substances List: Not listed
Edgewood Arsenal Number: EA 1250
Wiswesser Line Notation: 1Y&OPO&1&OY
Molecular formula: $C_7H_{17}O_3P$

Structural formula: $((CH_3)_2CHO)_2(CH_3)P{=}O$

DIMP is a liquid at room temperature with n_D^{20}=1.4112 (1), a bulk density at 25°C of 0.976 g/cc and a boiling point of 174°C (2, 3). Its vapor pressure-temperature behavior is closely approximated by the following empirical relationship (2, 3).

$$\text{Log P(mm of Hg)} = 9.8571 - 3105/T(^\circ K)$$

DIMP is best synthesized through the reaction of methyl iodide with triisopropyl phosphite (4, 5). Other methods are mentioned in the patent literature (6, 7, 8).

Very little is known of DIMP solubility in water. In studies of DIMP hydrolysis in acidic and basic solutions (9), 0.12 N or higher DIMP was used at temperatures above 80°C, indicating solubilities of above 11 g/liter in that temperature range. In DIMP studies at Southeast Research Institute (10), the solubility in water at 25°C was between 1 and 2 g/liter.

DIMP hydrolysis rates in water at 98, 90 and 80°C have been reported as 2×10^{-6}, 0.88×10^{-6} and 0.31×10^{-6} sec^{-1} respectively (11). The hydrolysis activation energy was estimated to be 26.9 Kcal/mole. These reaction rates can be used to predict hydrolytic behavior at 10°C, a temperature more representative of ground water in a temperate climate. The estimated rate is 3.2×10^{-11} sec^{-1}, corresponding to a hydrolysis half-life of about 687 years. In studies cited previously

(9), DIMP was among a series of alkylphosphonate esters whose hydrolysis characteristics were measured. In 1N HCl solution, rate constants of 1.74×10^{-4}, 2.81×10^{-4}, 4.79×10^{-4}, 8.53×10^{-4} and 8.56×10^{-4} sec^{-1} were determined at 88.9, 94.4, 99.7, 104.8 and 105.9°C, respectively (9). The acid hydrolysis appears to proceed by the S_N1 mechanism, since the rate of DIMP hydrolysis is greater than that of the lower alkyl phosphonate esters. Basic hydrolysis appears to proceed by the S_N2 mechanism, since the DIMP hydrolysis rate is less than that of the lower alkylphosphonate esters. Typical rate constants for 0.12 N DIMP in 0.2N NaOH solution were 1.53×10^{-4}, 2.29×10^{-4}, and 4.82×10^{-4} M sec^{-1} at 80, 90 and 100°C respectively. Basic hydrolysis at elevated temperatures is a convenient way to prepare the monoester, isopropyl methylphosphonate. In DIMP studies at Southeast Research Institute (10), the mono-sodium salt of DIMP was prepared by dissolving DIMP in 2N NaOH, heating to 50°C, followed by slow cooling to room temperature, with stirring applied throughout the process. About four days were required for completion of the hydrolysis reaction. It would appear that at room temperature and mildly basic conditions, hydrolysis of DIMP would be quite slow. DIMP is formed from sodium isopropyl methylphosphonate at 270°, but DIMP is also converted, in part, to trimethylphosphine oxide at this temperature (12). DIMP is decomposed almost entirely on short residence in a microwave plasma discharge (13); among the products are methylphosphonic acid, isopropyl methylphosphonate, phosphoric acid, isopropyl alcohol, and propylene.

DIMP forms a number of metal complexes in the absence of moisture (14, 15, 16).

DIMP does not appear to be a cholinesterase inhibitor (17).

ANALYTICAL METHODS

DIMP analysis by infrared and Raman spectra was reported by Meyrick and Thompson in 1950 (18). Strong infrared bands occur at 504, 983, 1008, 1248 (phosphonyl), and 2983 cm^{-1}, while strong Raman bands occur at 710, 1445, 2930 and 2985 cm^{-1}. Christol, Levy and Marty listed infrared absorptions at 987, 1015 and 1244 (phosphonyl) cm^{-1}. Moores (19) reported absorptions at 899, 1239 and 1314 cm^{-1}. The spectrum of DIMP was also studied by Lorquet and Vissart (20). Unfortunately, other alkylphosphonate esters have similar absorption bands.

Thin-layer and paper chromatography methods for DIMP have been studied (21). A 2:1:1 v/v solution of hexane-benzene-methanol or a 6:1:1 v/v solution of hexane-methanol-diethyl ether was used to develop the paper chromatogram. Spots were made visible with a spray of 1% cobalt chloride in anhydrous acetone, which detected DIMP and other phosphorous esters. These esters appeared at room temperature as blue spots, which could be distinguished by their relative R_f values. DIMP detection levels were not given.

Gas chromatography has been used to analyze DIMP in water with a flame ionization detector. Two methods are known; one developed by Shell Chemical Company (22) and adopted by the Colorado Department of Health (23) and one developed at Edgewood Arsenal (12). The Colorado Department of Health methodology (23) involves extraction of DIMP from water with chloroform. Three ml of chloroform suffices to extract 85-90% of DIMP from 200 ml of water. The glass chromatographic column was 5 ft long, ¼ inch in diameter, and filled with OV-17/Reoplex on 400 CRG. A 1 ppm solution of DIMP in chloroform was used as a standard.

The Edgewood Arsenal work (12) was oriented towards determining components of waste from demilitarized methyl isopropylphosphonofluoridate (GB). The waste is extracted with methylene chloride. The chromatography column was of glass, 6 ft long x ¼ inch in diameter and filled with QF-1 in 60-80 mesh Gas Chrom Q. GB could be detected by phosphorus flame photomety as 20 ppb (12); no limits were mentioned for DIMP or other compounds. Field ionization mass spectrometry (24) can be used to detect as little as 0.2 ppb (mole ratio), i.e., 10^{-9} g/liter.

The nuclear magnetic resonance spectrum of DIMP at 25 MHz was studied by Mavel and Martin (25).

MAMMALIAN TOXICOLOGY

No published information is available on the toxicity of DIMP to humans or experimental animals. Unpublished acute toxicity data (LD50) on experimental animals were obtained from the files at Edgewood Arsenal, and are summarized in Table E-1.

TABLE E-1

Summary of Acute Toxicity of DIMP

Animal Species	Route of Administration	LD50 (mg/kg)	Remarks	References
Mouse	Intraperitoneal	>250		26
Rat	Subcutaneous	>200		27
Rabbit	Subcutaneous	>100 <200		27
"	Intravenous	224	Local irritation 179-280 (19/20 confidence limits)	28
"	Dermal	>200	No irritation at application site	28

The toxicology of DIMP, including acute data, phytotoxicity and detoxification studies have been summarized in a fact sheet (2). No evidence was found that DIMP has been studied for carcinogenic, mutagenic or teratogenic activity in vitro. One report (17) stated that DIMP does not inhibit the enzyme cholinesterase, although no experimental evidence was given for the statement.

It is concluded from the data presented above, that DIMP is fairly toxic to experimental animals and could be irritating or corrosive to the eyes. The lack of complete data indicates the need for further studies to accurately evaluate the potential toxicity of DIMP. Recommendations for further toxicological studies were made (2) and these have been implemented through a USAMRDC contract with Litton Bionetics Inc., Falls Church, Virginia 22046.

An additional study on DIMP has been initiated (May 1975) in the Toxicology Division, Biomedical Laboratory, Edgewood Arsenal, APG, MD (29). This work includes a 26 week subacute study and a reproduction study in rats only.

ENVIRONMENTAL CONSIDERATIONS

No information was found as to DIMP behavior in soil and water, its effect on animals in the environment, or its transmittal in food chains. A USAMRDC contract study to determine the toxicity of DIMP to aquatic vertebrates and invertebrates has been initiated through Bionomics, E. G. & G., Inc., Wareham, MA 02571.

Plants

Evaluation of DIMP at Ft. Detrick during 1974-1975 indicated that DIMP could injure wheat and beans (Witchita wheat and Black Valentine beans) (30). In one test, treatment of wheat and beans (water solution to soil) with 10 ppm DIMP produced no effect on wheat, but gave a burning on edges of bean leaves. In a second test, treatment with 10 ppm or 40 ppm levels of DIMP resulted in tip burn of leaves on both wheat and beans at both levels. In other tests where DIMP and dicyclopentadiene (DCPD) were used together, there was an indication of additive or synergistic effects due to DCPD. DIMP may also be phytotoxic to sugar beets (31). In herbicidal screening tests at Ft. Detrick, rice, morning glory, bean, oat and soybean plants growing in pots in a greenhouse and sprayed with DIMP at 0.1 and 1.0 pounds per acre exhibited no injurious effects from the DIMP (32). A USAMRDC contract study to determine plant uptake and effects and soil retention of DIMP has been initiated through Aerojet Ordinance and Manufacturing Co., Downey, CA 90241.

EXISTING STANDARDS

No information available.

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APPENDIX F

CHLORATE (ClO_3^-) SALTS

COMPOUNDS CONSIDERED

Chloric acid; chloric acid, calcium salt; chloric acid, magnesium salt; chloric acid, potassium salt; chloric acid, sodium salt; chlorates; calcium chlorate (Chem. Abstr. before 1967); magnesium chlorate (Chem. Abstr. before 1967); potassium chlorate (Chem. Abstr. before 1967); sodium chlorate (Chem. Abstr. before 1967); Fekabit; oxymuriate of potash; potassium oxymuriate; Atlacide; soda chlorate; Val-Drop; De-Fol-Ate; E-Z-Off; Magron; Mc Defoliant; Ortho MC.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry Number and Toxic Substances List Number

Chloric acid, 7790-93-4; no TSL entry
Chloric acid, calcium salt, 10137-74-3; FN98000
Chloric acid, magnesium salt, 10326-21-3; F001750
Chloric acid, potassium salt, 3811-04-9; F005250
Chloric acid, sodium salt, 7775-09-9; F005250
Chlorate, 14866-68-3; no TSL entry

Chlorate (ClO_3^-) is one of the four oxy-chlorine ions. The conjugate acid is highly dissociated, with a pK_a of -2.7 (1). Table F-1 summarizes the physical properties of alkali and alkaline earth metal chlorates that would exist in common soils to which ClO_3^- had been added. Sodium chlorate is the best known of these, having been used as a herbicide for many years, and in leather, paper, and textile processing. Chlorate ion can be formed from the hypochlorite ion (ClO^-) (2). The reaction proceeds fastest in weakly alkaline or acid conditions. The reaction is best described as

$$2\ HClO + ClO^- \longrightarrow ClO_3^- + 2H^+ + 2Cl^-$$

TABLE F-1

Physical Properties of Common Alkali and Alkaline Earth Chlorates (1, 3)

Formula	Melting Point, °C	Density, g/cc	Solubility, g/100 ml H_2O(°C)
$Ca(ClO_3)_2 \cdot 2H_2O$	Loses water at 100°C	2.71	230 (25°C)
$Mg(ClO_3)_2 \cdot 6H_2O$	35	1.80	56.5 (18°C)
$KClO_3$	368	2.32	7.1 (20°C)
$NaClO_3$	248	2.49	79.0 (20°C)

In a 1956 study, Weintraub (4) mentioned factors such as pH, catalysts, temperature and ultraviolet radiation that affect the speed of the reaction. Chlorate ion undergoes relatively slow decomposition in solution when exposed to ultraviolet radiation. In a 1948 study by Farkas and Klein (5), a mercury lamp with emission from 190 to 260 nm and overall intensity of 10^{19} quanta/sec was used to irradiate 0.1M ClO_3^-. The solution absorbed light in the 190-225 nm wavelength region and underwent 6% conversion in 30 minutes at 30°C. Only O_2 and Cl^- ion were observed as reaction products.

The heats of solution for potassium chlorate and sodium chlorate are 9.89 and 5.18 kcal/mole, respectively (6). Infrared spectra have been obtained for chlorate salts (7, 8, 9). Chlorate ion absorbs only very weakly in the ultraviolet region (10, 11).

Chlorate ion was found to inhibit the enzyme catalase (12).

ANALYTICAL METHODS

Chlorate ion may be identified by means of paper chromatography (13, 14), thin-layer chromatography (15, 16, 17, 18), or paper electrophoresis (19, 20); the differentiation from bromate is particularly striking with anion exchange resin thin-layer chromatography (18). A spot test for chlorate ion in soil extracts using diphenylamine has been described (21). Another spot test for chlorate in soil extracts depends on conversion of Mn(II) to the violet Mn(III) (22). Analytical separation of chlorate ion from most other anions has been described by Taimni and Lal (23).

Chlorate ion is a relatively good oxidizing agent in acidic solution; the electrochemical half-reaction

$$ClO_3^- + 6H^+ + 6e^- \longrightarrow Cl^- + 3H_2O$$

has a potential of +1.48 volts at 25°C (3). This property has been used for a long time for chlorate analysis, wherein ClO_3^- is reduced to Cl^- by reaction with a suitable reagent, and a titrimetric Volhard analysis is carried out for Cl^- (24, 25, 26, 27). However, the sensitivity of this method is not sufficient for ppm detection and the need to subtract initial chloride concentrations makes the method unsuitable for chlorate in the presence of much chloride.

For titration of chlorate in the presence of hypochlorite interference, the latter can be removed by reaction with hot alkaline hydrogen

peroxide (28). A variety of titrants may be used for chlorate ion, all in acid solution, for example: ascorbic acid (28); arsenic trioxide (29); or ferrous ion (30) catalyzed by osmium tetroxide and back-titrated with bromate (29), ceric ion (30), or permanganate (31); reduction with iodide ion and back-titration of the formed iodine with thiosulfate (32, 33); reduction with vanadous ion and back-titration of excess vanadous ion with permanganate (34); and reduction with titanous chloride followed by back-titration with ferric ion (35). Mixtures of hypochlorite and chlorate have been analyzed by coulometric titration with ferrous ion, first, then with acidic titanous ion (36). Alternating-current cyclic voltammetry has also been used for chlorate analysis (37). The ability of the various chlorine-containing oxidants to oxidize iodide ion to iodine depends on the pH; by starting at nearly neutral pH and acidifying to successively lower pH's, one can titrate hypochlorite, chlorite and chlorate in turn (2, 38, 39).

A sensitive quantitative absorptiometric technique was reported by Urone and Bonde in 1960 (40), which involves reaction of ClO_3^- with *o*-tolidine in strong HCl solution. Concentrations of 0.5 to 10 ppm can be determined spectrophotometrically. For low concentrations, 448 nm light is recommended; for higher concentrations, 490 nm light is recommended. Procedures for chloride and nitrate interferences are indicated.

Trautwein and Guyon, in 1968 (41), reported an analytical method based on ClO_3^- interference with formation of the rhenium-α-furildioxime complex. In the presence of ClO_3^-, Sn^{+2} reduces perrhenate ion to Re^{+2}, which complexes with α-furildioxime. In the presence of ClO_3^-, Sn^{+2} reduces the chlorate, hence less complex is formed. The difference in complex color intensity at 532 nm can be calibrated for ClO_3^- content. The method can be used for 0-5 ppm chlorate; it is claimed (41) to be slightly more sensitive than the previously described method of Urone and Bonde (40). Levels of interfering ions are listed.

Reifenstein and Heinisch (42) devised a colorimetric method for determining chlorate residues in soil. This method depends on the oxidation of iodide ion to iodine at low pH and extraction of the iodine into carbon tetrachloride for spectrophotometric determination at 533 nm.

Ion pair formation with Nile blue, followed by extraction of the ion pair into 1,2-dichlorobenzene, permits colorimetric analysis down to about 0.4 ppm, though with potential interferences (43); some of these interferences can be masked by addition of mercuric ion. Similar analytically useful ion-pair formation was observed with crystal violet (44); interference by nitrate and perchlorate ions diminishes the potential usefulness of this reagent for analysis.

MAMMALIAN TOXICOLOGY

Human Exposures

According to Zahorsky (45), potassium chlorate was introduced to medicine as early as 1823 for the treatment of ulcerative stomatitis, a condition characterized by the appearance of shallow ulcers on the cheeks, tongue, and lips. Recommended doses for children ranged from 8 to 20 grains daily in small doses for several days. It was also recognized that large doses of potassium chlorate were poisonous, producing methemoglobinemia, destruction of red cells and toxic nephritis. Since potassium chlorate decomposes on heating to yield oxygen, and when mixed with sulfur, carbon, and other organic material in the dry state can be ignited or detonated by impact, the mistaken inference arose that chlorate in the body could serve as a souce of oxygen. Such metabolism does not occur and the chlorate ion is excreted unchanged in the urine with more than 95% of an oral dose being recovered with 36 hours (46).

Availability of potassium chlorate as an anti-infective drug through the 10th Edition of the National Formulary, 1955 (47), has resulted in a number of deaths through overdose or accidental substitution for other drugs. According to a review by Cochrane and Smith in 1940 (48), 155 poisoning cases had been reported by 1911, of which 116 were fatal. These authors cite an additional six cases, of which four were fatal. The single case they report in detail resulted from a mistake in the pharmacy in substituting potassium chlorate for potassium chloride. The subject had taken 30 to 35 grams, 10 grams per day with his food for 3 to 3.5 days. He died 10 days after the first 10 grams had been consumed. Signs of toxicity were classical for chlorate poisoning and consisted of pain in neck and legs followed the next day by abdominal pain, vomiting and diarrhea. The subject was cyanotic and his urine contained blood. Death was due to renal failure. Gordon and Brown 1947 (49) reported another case in which a woman of 61 had consumed as lozenges 20 five-grain tablets daily (6.5 grams) for 6 to 10 weeks to cure an imagined cancer of the tongue. This patient exhibited cyanosis when first seen but died 10 days later of renal failure even though the methemoglobinuria cleared up within the first 4 days. Renal failure is thought to be secondary to red cell destruction and methemoglobin collecting in the renal tubules. From such poisoning cases, the lethal dose of potassium chlorate is estimated to be in the 5 to 30 gram per person range. The sodium salt is probably equally toxic since Strzyzowski, in 1931 (50), reported the death of a man 8-10 hours after taking 20-30 grams of sodium chlorate. Two sodium chlorate suicides were described by Smith and Watson (51). Potassium chlorate was dropped from the National Formulary in the 11th Edition, 1960 (52), because of lack of efficacy and potential toxicity. The effect of the continued consumption of low levels of chlorates by man has not been investigated, although such consumption would have resulted from their use in toothpaste (53). The

taste threshold for chlorates is said by Mazaev to be 20 ppm (54). This was recommended as the permissible level for reservoir waters in the USSR.

Experimental Animals

The most extensive early animal studies were conducted by Richardson (55) because of the widespread use of chlorates in toothpaste at that time. He used 21 pigeons and 15 cats altogether and also conducted experiments on human blood and isolated frog esophagus preparations. The intact animal work is summarized in Table F-2.

No methemoglobin could be demonstrated in the pigeons and in surviving cats. Histologically there was kidney damage in all cats examined at dose levels higher than 0.05 g/kg/day. The sodium and potassium chloride controls also had some kidney damage, but not the fatty degeneration shown by the cats given chlorate.

The effect of increasing concentrations of potassium chlorate on the beating cilia of the frog esophagus was matched by equal molar concentrations of sodium chlorate and sodium chloride. Slowing of the cilia-mediated transport is therefore a non-specific effect.

Methemoglobin is formed in vitro when a chlorate salt is added to blood (56). Methemoglobin is also said to catalyze oxidations by chlorate (56). The in vitro studies with human blood, in retrospect, may have been incorrectly interpreted. Erythrocytes normally contain small amounts of methemoglobin in a steady state resulting from oxidation of ferrous (hemoglobin) to ferric (methemoglobin) on the one hand, and the reduction by two enzyme systems - methemoglobin diaphorase and methemoglobin reductase on the other (57). Thus, chlorates may interfere with one or both of the methemoglobin reducing systems--allowing the methemoglobin level to increase to dangerous levels. In dogs, the lethal dose of sodium chlorate was approximately 2 g/kg (58).

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

The behavior of chlorate ion in soil has been studied due to the employment of chlorate in herbicides. However, much of the work is old and lacks detail as to the actual chlorate content in soil, relying instead upon the observed phytotoxic effect.

Chlorate can be moderately persistent in areas where leaching does not occur. Sigler and Andrews (59) cited an example in Texas where

TABLE F-2.

Animal Toxicity Studies - Chlorate Salts

Preparation	No.	Daily Dose Level	Route	Remarks
Pigeons	4	5%	Drinking water	Death in 3 days
	5	1%	Drinking water	13-53 days, 20% weight loss; 2 died
	4	0.1 to 0.5%	Drinking water	30-35 days, no weight loss
	1	1 g/kg	I.M.	Death in 8 hours
	2	0.5 g/kg	I.M.	27 days no effect
	1	0.25 g/kg	I.M.	26 days no effect
	1	0.25 g/kg (10%)	I.M.	36 days no effect
	1	0.5 g/kg (10%)	I.M.	36 days no effect
Cats	1	1.0 g/kg (5%)	I.M.	Death in 8 hours
	3	0.5 g/kg (5%)	I.M.	Death in 2, 2, and 10 days
	4	0.05 to 0.25 g/kg (5%)	I.M.	28-32 days, 1 cat died of pneumonia on the 24th day, otherwise no effect
	3	0.05 to 0.2 g/kg (10%)	I.M.	25-32 days, no effect
	1	1.0 g/kg NaCl	I.M.	20 days, severe weight loss
	2	0.5 g/kg NaCl	I.M.	7-12 days, weight loss, pneumonia, death
	1	0.5 g/kg K Cl	I.M.	10 days, severe weight loss

800 lb/acre application of $NaClO_2$ was effective for three to five years. Other researchers have reported two or three years persistence (60, 61, 62, 63). Ungerer (21) observed a decrease in soil chlorate concentrations in the course of pot experiments with mustard plants, and concluded that the effect was due to uptake and reduction by the plants.

In 1933, Loomis, *et al.* (64), related chlorate movement through subsurface levels of soils to the phytotoxic effect to corn grown in soils collected from the different levels. An October 1930 application to the surface required seven months in relatively dry weather to reach the three-to-four-foot level in concentrations enough to damage corn slightly. By November 1931, chlorate phytotoxicity in the zero-to-one-foot level decreased to the extent that corn was only slightly damaged.

Loomis, *et al.* (64), also studied temperature and moisture effects on chlorate phytotoxicity. After chlorate-treated soil had been stored for 10 weeks at 3°C, the initial chlorate phytotoxicity was still observed. At elevated temperature (up to 40°C), phytotoxic effects decreased earlier in the storage period. Increased soil moisture also hastened decreases in chlorate phytotoxicity. A 1957 report by Tovorg-Jensen and Larsen (65) indicated that half the chlorate applied to an aerated soil was reduced in 5 months at 20°C. Chlorate applied to a wet clay soil with no sub-soil oxygen underwent only 8% reduction in 5 months.

In part, the disappearance of chlorate from the soil can be explained by the fact that at moderate temperature and moisture, microorganisms can use chlorate as an oxygen supply. Thus, Bryan and Rolich (66) demonstrated this effect in settled sewage samples. They even suggested chlorate addition to perform a BOD_5 test, with the chloride ion increase as the index of oxygen uptake.

Leaching of soils to remove chlorate ion (at least to below phytotoxic levels) has been demonstrated by Crafts (60), who used 40 cm of water for a clay loam and a fine sandy loam soil. Tovborg-Jensen and Larsen (65) removed 95% of applied chlorate by 20 cm of water on sandy soil, 40 cm on a humus soil, and 30 - 70 cm on clay soils. An intermittant leaching routine was employed.

Animals

Mammals: A study in eight beagle dogs (67) showed that daily administration of 200 to 326 mg/kg of sodium chlorate by stomach tube for five days resulted in moderate to severe hemolytic anemia without methemoglobin production except in one dog which died after four days. Additional animal toxicology data add little to complete understanding of chlorate toxicity, but are tabulated in Table F-3.

Fish and Other Aquatic Organisms: The toxicity of potassium chlorate was

TABLE F-3

Animal Toxicology Data - Chlorate Salts

Species	Salt	Dose Level	Route	Duration	Effect	Ref.
Rabbit(a)	K	60-120 mg/kg/day	oral	6 weeks	none	69
Rabbit(a)	K	185-738 mg/kg/day	oral	6 weeks	none	69
Rabbit	K	1 g/kg/day	oral	4 weeks	none	70
Rabbit	Na	1 g/kg/day	oral	4 weeks	none	70
Rabbit	Na	5 g/rabbit	oral	daily	none	71
Rabbit	Na	10 g/rabbit	oral	single dose	death	71
Rabbit(b)	Mg	Not stated	oral	7th day gestation	see note	72
Rat	K	1 g/kg/day	oral	4 weeks	none	69
Rat	Na	1 g/kg/day	oral	4 weeks	none	69
Rat(c)	Mg	3 g/kg/day	oral	6 months	see note	73
Chicks	Na	5 g/kg	oral	single dose	lethal dose	74
Sheep	Na	2.06-2.5 g/kg	oral	single dose	lethal dose	74
Sheep	Na	15 g/head	oral	3 days	death	71
Sheep	Na	30 g/head	oral	2 days	death	71
Sheep	Na	7.5 g/head	oral	20 days	transient diarrhea only	71

TABLE F-3 (Cont'd)

Species	Salt	Dose Level	Route	Duration	Effect	Ref.
Cat	Na, K	0.5 g/kg/day	oral	unstated	none	75
Cat	Na, K	1.35-1.94 g/kg	oral	single dose	death, methemo-globinuria	75
Goat	Na	Small amounts	fodder	continuous	more sensitive than sheep	76
Dog(d)	K	0.5 g/kg	oral	single dose	none	77
Horse	Na	120-130 g/head	oral	single dose	severe toxicity	71
Horse	Na	60 g/head	oral	single dose	slight methemo-globin	71

(a) 60-80% excreted in urine unchanged.

(b) Reports intrauterine deaths and resorptions in rabbits held at 36.6°C but not at 21°C.

(c) 55-70% excreted in urine within 6 hours unchanged.

(d) 61 offspring at 2 days examined histologically exhibited pulmonary and cerebral edema, focal hemorrhage in lungs, thus indicating placental transfer.

tested (68) on several species of fish, i.e., carp (*Cyprinus carpio*), *Lepomis gibbosus*, *Carassius carassius*, *Carassius auratus gibelio*, *Scardinius erythrophthalmus*, and *Rutilus rutilus carpathorossicus*. Concentrations above 1000 ppm were lethal. Concentrations of potassium chlorate higher than 100 ppm were lethal to plankton (68).

Microorganisms: Coliform bacteria, such as *E. coli*, which ferment lactose to acid and gas, are more susceptible to chlorate toxicity than are the true lactic acid bacteria used to make cheese (78, 79). These lactic acid bacteria ferment lactose, producing lactic acid, but little or no gas. The addition of 0.002% $KClO_3$ to raw milk selectively inhibits coliform growth and, thereby, prevents unwanted gas formation during cheese-making (79). One percent solutions of chlorate, though lethal to plants, are tolerated by numerous bacteria and fungi (80). It has been demonstrated (66) that 1000 ppm chlorate added to settled sewage samples was not toxic to the over-all microbial flora, and that chlorate could be used by the microbes as an oxygen source. The green alga, *chlorella fusca*, reduces chlorates to non-toxic chloride, following Michaelis-Menten kinetics (81). It has been shown that chlorate, as well as other inorganic ions, can be reduced by many diverse soil microorganisms, and that these reductions may influence the solubility, availability, or toxicity of elements in soil (82). A number of workers have shown that chlorate is toxic to certain fungi (83, 84) and algae (85). In these cases the basis for chlorate toxicity seems to be the same. Chlorate, though non-toxic itself, is reduced to toxic chlorite (85). Chlorate reduction occurs when cells are utilizing nitrate as a source of nitrogen. The induction of nitrate reductase by nitrate ion triggers the toxic reaction because this enzyme will also reduce chlorate to chlorite. Chlorate and nitrate appear to compete for the enzyme. The fungus, *Aspergillus oryzae*, for example, is only weakly inhibited by chlorate in the presence of ammonium or nitrite ions, but strongly inhibited in the presence of nitrate. The same pattern of toxicity in the presence of nitrate is observed with another fungus, *Aspergillus nidulans* (83). Interestingly, nitrate reductase negative mutants of this fungus become chlorate-resistant in the presence of nitrate.

Plants

Sensitivity of plants to chlorate depends primarily upon the species of plant (86, 87, 88, 89); nitrates, arsenites, and borates in soil (90, 91); type of soil (90, 92, 93); and concentration of chlorates (86, 94, 95). Temperature, pH, and light have also been reported to influence sensitivity of plants to chlorates, as well as age and differing rates of development (80, 87, 89, 93, 96, 97, 98).

Sublethal concentrations of chlorate in plants produce a characteristic mottling along with chlorosis and over-all stunted growth (99, 100, 101).

Starch reserves, catalase activity, and susceptibility to frosts are also known to be altered (100). When the concentration of chlorate is lowered, plants may recover from sublethal amounts and show no permanent effects (100) and, in fact, low chlorate concentrations may be stimulatory to certain species (102, 103).

Since chlorates are not specific for weeds (99), wide variation in susceptibility occurs among species. Some examples of plants in which chlorates in soil have been shown to influence growth and development are witch weed (104), hoary cress (100), morning glory (100), alfalfa (86), beans (86), oats (86,105), zinnias (86), wheat (96), radish (106), sudan grass (106), winter rye (105), barley (105), and tomato (99). Toxic concentrations range from 6 ppm (witch weed) (104) to over 2690 kg/ha (approx 1000 ppm) for certain grasses and broadleaf plants in test plots where ground cover was reduced by approximately 90% (107). Concentrations exceeding 28.6 mg/liter were harmful to wheat (91, 94, 108). Many plants have a reduction in growth upon treatment with 10 ppm sodium chlorate (86). There was a 50 percent reduction in growth of oat plants growing on Stockton adobe clay soil containing 36 ppm sodium chlorate (90). Some plants appear more sensitive when under water "stress" and during spring growth (100), and older leaves of excised shoots of morning glory plants are apparently killed before younger ones (100). Two species of aquatic plants showed some survival even after eight days in a 1% chlorate solution (109). Fungi and bacteria were also observed to endure concentrations which were known to kill higher plants within a few days (80).

Crafts (60, 90, 92) studied the phytotoxicity of chlorates in 80 California soils. Toxicity varied over five times from soil to soil, with highest toxicities generally in coarser soils and lowest toxicities generally in alluvial soils (92, 100). The chlorate "holding" capacity of the soil determines if there will be a chlorate concentration sufficient to injure plants (90). $NaClO_3$ applied at the rate of 800 lbs. per acre is sufficient to sterilize soil for three to five years (59). The presence of nitrates in the soil seems to inhibit absorption of chlorates by some plants (86, 91, 93, 108, 110).

Chlorate is absorbed through all plant surfaces, particularly the roots. Cork layers tend to retard absorption. Scharrer and Schropp (111) have indicated, through germination studies with wheat, rye, barley, and oats, that the injurious forms of chlorine in plants could be ranked as $NaClO_4 > NaClO_3 > CaCl_2 \cdot 6H_2O > NaCl$.

Bioaccumulation: Chlorates are non-selective systemic poisons and may be cumulative in plants until concentrations in cells are high enough to cause death of cells (100).

Degradation: Chlorates appear to be competitive with nitrates as a substrate for the enzyme nitrate reductase (81, 112). Therefore, plants may

reduce chlorate to the phytotoxic chlorite ion (85, 96). This toxicity has been suggested to be due to the irreversible inhibition of the enzyme by chlorite or some decomposition product of chlorite (85). However, this mechanism may not work for all plants. For example, in the fungus, *Aspergillus nidulans*, chlorate appears to be toxic not because it is converted into chlorite but because it interferes with the normal breakdown of organic nitrogen compounds to provide sources of nitrogen (83).

Nitrate on the other hand, is a reversible competitive inhibitor of chlorate reduction (85) and as such may account for the partial reversal of chlorate effects if there is excess nitrate concentration in the soil (91, 93, 110). It should be mentioned, however, that one report involving field studies indicated that nitrates gave poor reversal of chlorate toxicity (94).

Food Chain

Chlorates are non-selective poisons to plants, in which they may be cumulative until death of tissue (100). There is no evidence for their accumulation in animals.

EXISTING STANDARDS

No information available.

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APPENDIX G

WHEAT RUST

ALTERNATIVE NAMES

Wheat stem rust (Puccinia graminis tritici); stripe rust (Puccinia glumarum); wheat leaf rust (Puccinia rubigovera); stem rust; cereal rust; Puccinia recondita; Puccinia striiformis; 15B strain; Puccinia graminis. Defense Department Symbol: TX.

PHYSICAL AND BIOLOGICAL PROPERTIES

Approximate specific gravity 0.6; size 20-25 microns; weight 2.5×10^{-9} grams; spores drop at the rate of 1 meter/minute in still air; in wind they can be blown for great distances dependent on wind speed, updrafts; in spring through fall of 1951 race 15B of stem rust traveled at least 4000 miles; in 1950 it spread over an area of 2,000,000 square miles which was for at least 10 years previously uninfected by this strain (1).

In the northern states the barberry plant (Berberis vulgaris, also known as Barbaris vulgaris) is necessary for wheat rust overwintering. In places where this plant has been eradicated the "rust" problem has subsided. However, with staggered growing seasons from north to south, the disease may spread north from southern refugia where it can overwinter in the absence of the barberry. In the north, the thick walled spores (teliospores) survive only if they land on barberry plants. Aeciospores produced in the spring reinfect the wheat crop, producing uredospores (red rust) which are the asexual phase and therefore remain stable as a strain. New strains are produced in the sexual phase in the barberry plants. Some 240 parasitic strains of stem rust are known, of which only about 12 are prevalent. Strain 15B which became prevalent in 1950 attacked all resistant varieties of wheat known at that time (1).

ANALYTICAL METHODS

Viability of spores can be tested by germination. For germination, the optimal temperature is 70-75°F, with a dew duration 3-4 hours or more; germination is possible from 50-80°F; average germination time is 10 days under optimal temperature and dew duration.

MAMMALIAN TOXICOLOGY

No information is available for either humans or experimental animals.

ENVIRONMENTAL CONSIDERATIONS

Wheat plants can be attacked and injured. Uredospores are responsible for rust disease appearing in epiphytotic proportions (2). The reduction in wheat yield from "rusted" plants is primarily due to rust organisms' growth on the plant's leaf and stem where it absorbs photosynthate from the plant. This infection and removal of plant sugars weakens the plant and leaves no sugar for deposition in the wheat kernels, which results in shriveled and shrunken grains in place of normal grains at harvest time. Rusted plants lodge (fall down) from their weakened condition, and poor development of root systems does not allow the plants to take up enough water in dry weather. The total damage to the wheat plant (reduction in yield) depends upon the age of the plant at the time of infection, with younger plants being more severely injured. The Puccinia graminis tritici organism infects only wheat plants and the barberry bush (alternate host for sexual reproduction). The uredospores of wheat rust endanger no plants except wheat.

Once infection of wheat begins, it continues logarithmically as long as conditions (mainly temperature and humidity) are favorable for infection of susceptible wheat (3, 4). Winds continuously carry the spores to new wheat fields. The quantity of spores needed to initiate an epidemic depends upon the infection rate of the rust organisms (dependent on plant resistance and climatic conditions). High humidity and temperatures of 5-20°C favor wheat rust spore germination on wheat leaves.

There appears to be no hazard to wheat crops of the U.S. or Canada from rust spores stored at RMA. Uredospores are unable to survive the winter in cold climates of the cereal belt (4, 5, 6, 7). Epidemics of rust infections at the national level from uredospores generally start in Mexico each year and are carried northward by winds throughout the U.S. and into Canada (4, 5, 6, 7). Longevity of the uredospore depends upon storage temperature and moisture content of the spores. Reduction of spore moisture to 10 percent and holding at 4°C enable them to retain germinability up to 2 years (8). Vacumn dried spores stored in absence of oxygen or water will survive up to 5 years (8). Other studies have indicated a 98 percent decrease in germination of hydrated and unhydrated uredospores stored at 30°C for 5 weeks (9). Indications are that viability of wheat rust uredospores in storage depends upon the particular rust organism, but generally there is a loss of 24-90 percent germination in 150 days (10). The half-life of processed spores (stored under anerobic condition at 4°C) is 36 months while for unprocessed spores (no special storage conditions) it is less that one month (10).

EXISTING STANDARDS

Not applicable.

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APPENDIX H

ARSENIC

COMPOUNDS CONSIDERED

Arsenic Acid (H_3AsO_4)
Arsenic Acid, Disodium Salt - Sodium arsenate dibasic, anhydrous
Arsenic Acid, Iron (3+) Salt
Arsenic Acid, Magnesium Salt - Magnesium arsenate
Arsenic Chloride - Arsenic butter, Arsenic (III) chloride, Arsenic trichloride, Arsenous chloride, Fuming liquid arsenic
Arsenic Pentoxide - Arsenic acid anhydride, Arsenic oxide
Arsenic Trioxide - Arsenic (III) oxide, Arsenic sesquioxide, Arsenous anhydride, Arsenous oxide, Arsenous acid anhydride, White arsenic
Arsenious Acid, Sodium Salt - Sodium arsenite, Sodium metaarsenite
Calcium Arsenate
Lead Arsenate (basic)
Arsenate (AsO_4^{-3})
Arsenite (AsO_2^{-1}) and (AsO_3^{-3})

PHYSICAL AND CHEMICAL PROPERTIES

CAS Registry Number and Toxic Substances List Number

Arsenic Acid (H_3AsO_4); 7778-39-4; CG0700O
Arsenic Acid, Disodium Salt (Na_2HAsO_4); 7778-43-0; CG08750
Arsenic Acid, Iron (3+) Salt ($FeAsO_4$); 10102-49-5; No entry
Arsenic Acid, Magnesium Salt ($MgHAsO_4$); 10103-50-1; CG10500
Arsenic Chloride ($AsCl_3$); 7784-34-1; CG17500
Arsenic Pentoxide (As_2O_5); 1303-28-2; CG22750
Arsenic Trioxide (As_2O_3); 1327-53-3; CG33250
Arsenious Acid, Sodium Salt ($NaAsO_2$); 7784-46-5; CG36750
Calcium Arsenate ($Ca_3(AsO_4)_2$); 7788-44-1; EV94500
Lead Arsenate (basic) ($PbHAsO_4$); 7784-40-9; OF85750
Arsenate (AsO_4^{-3}); 15584-04-0; No entry
Arsenite (AsO_2^{-1}); 17306-35-3; (*)
Arsenite (AsO_3^{-3}); 15502-74-6; (*)

*The *Toxic Substance List* (1) cites CG-61250 for arsenite; the species is not cited.

Physical properties of $AsCl_3$, the arsenic oxides, and selected arsenates are listed in Table H-1. The discrepancy in these data, between different literature sources, is due to variable amounts of bound water in the substances tested; or the structures involved are not as simple as the molecular form cited. Very little physical data have been found for the arsenite salts, the most important of which is sodium arsenite ($NaAsO_2$ or Na_3AsO_3)*. In information sheets presented by Ottinger et al. (2), most arsenites are considered soluble in water.

Arsenic trioxide is the starting material for the production of agricultural pesticides and herbicides. The commercially used arsenates are those of lead and calcium. Sodium arsenate, generally as $Na_2HAsO_4.7H_2O$, is used in many experimental studies due to its high solubility in water.

The aqueous chemistry of these compounds is quite complex; arsenic behaves similarly to phosphorus in water in this respect. Arsenic chloride decomposes in water to form HCl and $As(OH)_3$ (3). Above the solubility point, As_2O_3 is precipitated. In basic solution, As_2O_3 is more soluble than indicated in Table H-1 and exists in complex forms such as $(AsO(OH)_2)^-$, $(AsO_2(OH))^{-2}$, or AsO_3^- (3). The AsO_2^- ion is not formed under these conditions, although alkaline arsenites are recovered from evaporated solutions of As_2O_3 in alkali-base solutions (3). Arsenite solutions are somewhat basic, as the conjugate acid, $HAsO_2$, has a pK_a of 9.2 at 25°C (3). They can be readily oxidized to arsenate, as the half reaction

$$AsO_2^- + 4OH^- = AsO_4^{-3} + 2H_2O + 2e^-$$

has a +0.67 volt potential at 25°C (3).

Arsenic acid can be prepared from the oxidation of As_2O_3 in concentrated nitric acid (3). Arsenic acid is moderately strong with pK_1, pK_2, and pK_3 values of 2.2, 6.9 and 11.5, respectively (3). Thus, mono-, di- and tri-basic salts can be formed. Upon heating, arsenic acid is converted to hydrated As_2O_5, and above 170°C, anhydrous As_2O_5 (3). Many of the evaporated salts occur as the AsO_3^- arsenate as opposed to AsO_4^{-3} in solution.

Rather vigorous conditions must be applied to reduce arsenic compounds chemically. In a study reported by Braman and Foreback (7), quantitative reduction of As(V) ion to As(III) ion was accomplished with sodium cyanoborohydride after pH adjustment to 1 or 2. As(III) was reduced to arsine (H_3As) with sodium borohydride at pH 1-9, but not As(V).

*The naming of arsenic compounds is somewhat imprecise. For example, the mono-, bi- and tribasic sodium arsenite salts are called sodium arsenite. A similar situation exists for the arsenate salts.

TABLE H-1. Selected Physical Properties of Arsenic Chloride, Arsenic Oxide, and Arsenates (2, 3, 4, 5, 6)

Compound	Melting Pt, °C	Boiling Pt, °C	Density, g/cc	Solubility, g/100g H_2O*	Vapor Pressure Data
$AsCl_3$	-16.2	103.2	2.17	Decomposes	1 mm Hg at -5°C, 10 mm Hg at 26°C, 100 mm Hg at 71°C**
As_2O_3	Sublimes, 193	457.2	3.74†	2.04 (25)	1 mm Hg at 212°C, 10 mm Hg at 259.7°C, 100 mm Hg at 332.5°C
As_2O_5	Decomposes, 800	---	4.32	39.7 (25)	----
$AlAsO_4$	---	---	3.25	2.1×10^{-5} (25)††	
$Ca_3(AsO_4)_2$	1455	---	3.62	0.0139 (25)	
$Na_2HAsO_4.7H_2O$	125	Loses water at 100	1.87	61 (15)	
$FeAsO_4$	---	---	3.18	1.5×10^{-7} (25)††	
$PbHAsO_4$	Decomposes, 720	---	5.79	---	
$MgH(AsO_4).7H_2O$	---	Loses $5H_2O$ at 100	1.94	---	

*Temperature in °C in brackets.
**Calculated from pressure-temperature relations presented in reference 4.
†Condensed from sublimed material. Natural mineral forms have different densities and melting points.
††Calculated from K_{sp} in reference 5. K_{sp} $AlAsO_4 = 1.6 \times 10^{-16}$, K_{sp} $FeAsO_4 = 5.7 \times 10^{-21}$.

Biochemical systems transform arsenic* between different valence states or convert inorganic arsenic compounds to organic compounds. Some of these systems occur in environmental situations and are discussed in the "ENVIRONMENTAL CONSIDERATIONS" section.

The marine alga, L. calcareum, has been reported capable of removing arsenic from solution (8). The alga has a high $CaCO_3$ content, and the mechanism may be one of precipitation of a calcium arsenate from solution rather than a bioconversion. Another alga, C. fragilis, exhibits similar behavior (9). Pilson (10) reports that the coral Pocillopora verrucosa converts As(V) to As(III) in seawater medium.

A number of bacteria and fungi are capable of methylating and reducing arsenates or arsenites to the rather poisonous gases arsine, dimethylarsine, and trimethylarsine (11). Digestion of inorganic arsenic compounds results in their conversion through the intermediate methylarsonic acid ($CH_3AsO(OH)_2$) to dimethylarsinic acid or cacodylic acid ($(CH_3)_2AsOOH$) (12). These compounds are also intermediates in the conversions cited above (11).

ANALYTICAL METHODS

Several reviews (13, 14, 15) discuss the merits of the many techniques available for arsenic determination. The method selected depends on the time and equipment available, the material to be analyzed, the concentration range of interest, and the accuracy and precision required.

Depending upon the sample analyzed and the assay requirements, trace analysis for arsenic may require one or more of the following steps: dissolution; extraction; separation; concentration; and analysis. Dissolution is required for samples with organic or insoluble inorganic arsenic. These samples may be treated by wet ashing, dry ashing, oxygen combustion (Schöniger method) or fusion to bring the arsenic into solution. Soil samples contain various forms of arsenic of possible interest to the analyst. A number of methods are available for extracting these arsenic species from soil (16). Once the arsenic has been brought into solution, it may be necessary to separate it from other species that may interfere with the analysis. Preconcentration may be necessary to bring the arsenic concentration within the useful range of the analytical procedure employed. This separation and concentration may be accomplished by volatilizing and trapping arsenic as arsine, coprecipitation and adsorption of arsenic, or liquid-liquid extraction of arsenic from solution.

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*For the sake of brevity, the term "arsenic" is used in much of the environmental literature to refer to compounds containing the element. This is done because it is rarely known under uncontrolled situations which compounds are present, and most analyses are in terms of total arsenic.

Certain of the analyses presented are specific for one valence state of arsenic. For example, polarography measures As(III), while the arsenomolybdate method measures As(V). Many of the articles cited, and others reviewed but not cited, are based on variations of the preparatory steps needed to convert a sample to a state in which it is ultimately analyzed. Methods which ultimately convert arsenic to arsine are "total arsenic" methods. Others, by proper conversion of valence or lack thereof, can measure either or both As(III) and As(V). Much of the current work underway is oriented towards more specific measurements, especially of the methane-arsenic compounds.

The analyst has a choice of eight general analytical approaches which can be employed for arsenic (Table H-2).

Conventional atomic absorption (AA) spectroscopy is probably the simplest and fastest method available for aqueous arsenic. The sample is simply aspirated into the flame and its absorbance at 193.7 nm is measured and compared to standard samples. However, this method is subject to many common interferences and, without preconcentration, lacks the sensitivity required for most environmental analyses. Boat or cup techniques using samples concentrated by extraction can lower the detection level to 20 µg/l.

A considerable improvement in sensitivity of AA occurs when the arsenic is converted to arsine. This serves to concentrate arsenic and remove interfering ions. The arsine can be trapped in aqueous solution and analyzed by conventional AA (20, 21); or, it can be injected directly into the flame (19, 22, 23, 24, 25, 26, 68, 69). EPA recommends an arsine-flame method (24, 70) for the analysis of arsenic. The arsine methods have detection limits in the sub-ppb level.

The flameless AA methods provide greater sensitivity but are often more complicated and in most cases handle only small samples. Such techniques often require pretreatment and/or preconcentration but are useful for sub-ppb concentrations of arsenic. Several methods involve the detection of arsine: either by passing the gas through a heated "Vycor" tube (29), or graphite furnace (22), or through a quartz tube (30, 31). These last two references describe automated methods, one for water samples (30), the other for particulates collected from air on glass fiber filters (31). One method (28) measures arsenic in atomized ashed samples in a heated carbon tube. It handles 20-µl samples and has detected 6 to 315 ppb As in water samples.

An indirect method involves the precipitation of arsenomolybdic acid and analysis of the precipitate for molybdenum at 313.3 nm. This eliminates the interferences found at the wavelength used for arsenic and concentrates the sample for better sensitivity. The indirect method can be used for samples having 0.01 to 1 mg/l As.

TABLE H-2. Analyses for Arsenic

Method	Detection Limit (Useful Range)*	Comments	References
Atomic Absorption			
Conventional Flame	0.15 mg/l (0.5-50 mg/l)	Several interferences	14, 17, 18
Arsine-Flame	0.1 µg/l (0.1-50 µg/l)	EPA proposed method	19, 20, 21, 22, 23, 24, 25, 26
Flameless	0.005 µg/l (6-315 µg/l)		22, 27, 28, 29, 30, 31
Indirect for Mo	<0.01 mg/l (0.01-1 mg/l)	Arsenomolybdate ppt. formed	32, 33, 34, 35
Atomic Emission	0.1 µg/l (1-1000 µg/l)	Preconcentration required	7, 36, 37, 38, 39, 40
Activation Analysis	0.01 µg (0.01 µg/l-500 mg/l)	Expensive, Lengthy	41, 42, 43, 44, 45, 46, 47
Chemical Methods			
Gutzeit	1 µg	Color strip test, Color at 535 nm	48, 49
Silver Diethyl-dithiocarbamate	1 µg/l (10-300 µg/l)	"Standard" Method, color at 560 nm, good precision and accuracy	24, 32, 48, 50, 51, 52, 53
Arsenomolybdate	0.02 mg/l (0.02-1.2 mg/l)	Color at 840 nm	54, 55

TABLE H-2. Analyses for Arsenic (Cont)

Method	Detection Limit (Useful Range)*	Comments	References
Electrochemistry			
Direct Pulse/Single Sweep polarography	20 µg/l		56
Stripping Voltametry	0.1 µg/l (0.5 µg/l-100 mg/l)		57, 58
Differential Pulse polarography	0.1 µg/l (0.5 µg/l-60 mg/l)	Use for "dirty" systems questionable	58, 59, 60
X-Ray Fluorescence (with preconcentration)	1 mg/l (1-1000 mg/l) (5-500 µg/l)	Simple, non-destructive	61, 62, 63
Gas Chromatography	0.4 ng	Conversion to volatile compound required (see text)	14, 64, 65
Mass Spectroscopy	$\leq$ 3 µg/l	Expensive Equipment	66, 67

*Liter volumes are used throughout although actual sample sizes are usually of less volume. In some methods, the detection limit is based on As mass and not concentration considerations. The useful range may be expanded by a sample concentration.

Conventional atomic emission spectroscopy lacks the sensitivity required for trace analysis of environmental samples (14). Emission analysis has been used to analyze natural waters for arsenic over the range of 0.2-1 mg/l (37). However, with better excitation sources and preconcentration, this method can be used to analyze ppb levels of arsenic. Plasma excitation of arsine (40) was used at 235.0 nm to determine arsenic with a detection limit of 5 ng. The method was limited by the arsenic background of the reagent blank. A d.c. arc method with arsine (36), could detect 1 ppb concentrations. Selective preparation and volatilization followed by emission analysis enabled one group (7) to determine the concentrations of As(III), As(V), methylarsonic acid and dimethylarsenic acid in a variety of samples. The detection limits were 0.05 ng for inorganic arsenic and 0.5 ng for the organic acids. Anion exchange concentration of arsenic followed by d.c. arc analysis (38) was able to detect 0.001 ppm concentrations in one-liter samples with a precision of $\pm$ 0.001 ppm.

Neutron activation analysis is one of the most sensitive methods available and with the proper equipment can be non-destructive. However, it is also expensive (requiring a strong neutron source and sophisticated equipment) and time-consuming (hours to days required for hot radiation decay). Activation analysis depends on the reaction $^{75}As\ (n,\gamma)\ ^{76}As$, where As^{76} has a half-life of 26.5 hours (14). As^{76} decays to Se^{76} by β^- emission and γ radiation; therefore, active samples can be analyzed by β^- counting and/or γ-spectroscopy. The analytical procedure involves irradiation, cooling to allow dangerous or interfering radiation to decay, sample treatment (separation and/or concentration) when necessary, and analysis. This method has been employed on a variety of samples. Arsenic contents of 2.6 to 93 ppm were determined from 400-year old human remains (43); the procedure was deemed capable of detecting 10^{-8} to 10^{-9} g As. Several procedures for analysis of biological samples have been reported (44, 45, 47, 71, 72); the sensitivity of such procedures is about 1 to 20 ng As/g sample. Soil can also be analyzed with a reported 0.005 ppm sensitivity (41). Natural waters are also amenable to neutron activation (46, 71, 73) with 1 µg/l measured in Rhine River water (73) and 2-38 ng/l measured in melted glacial ice (46).

A radiometric filter-spot technique has also been described (74), whereby alpha particles from U disintegration of $NH_4UO_2AsO_4$ salt precipitate are counted. It is considerably less sensitive than neutron activation.

A number of chemically-based methods are available. The Gutzeit method (48, 49, 75, 76, 77) relies on generation of arsine and reaction with $HgBr_2$ to produce a color strip. The length of the strip is proportional to the mass of arsenic. It is relatively easy to perform, but its precision is limited by the reproducibility of strips. One improvement that has been made is an X-ray spectrographic reading method (49).

An improved method measures the color at 560 nm produced by the reaction of arsenic with silver diethyldithiocarbamate (SDDC). A modified Gutzeit method using a SDDC strip indicator was developed for the Army (76). However, SDDC finds its widest use as a wet-colorimetric method, and is the standard method for water and wastewater (24, 50). Based on the method of preparation, it has been used for water samples (32, 48, 51, 78), plant samples (48), soil samples (52), and air samples (53, 79). One study (56) has criticized the SDDC method, in that interferences in certain natural waters can cause color enhancement or arsine suppression.

The arsenomolybdate method is another common colorimetric method (16, 54, 55, 80, 81, 82, 83, 84). It relies on measurement of the color produced at 840 nm by arsenate ion and can detect concentrations as low as 0.02 ppm. A closely related method for measurement of arsenate in natural waters has been described (85), whereby combined phosphate and arsenate form molybdate complexes which are measured at 865 nm. A portion of the sample is reduced to convert arsenate to the non-complexing arsenite. Arsenate is computed by difference between the intensity readings of the two portions. In ocean water samples with 0.013 µg/l phosphate, 0.0013 µg/l arsenate could be determined with reasonable accuracy. Another modification (86) involves extracting the complex formed with arsenate and ammonium molybdate/ammonium vandate into isobutyl alcohol and adding brilliant green dye. Color is measured at 470 nm. As(III) can be oxidized to As(V), and the analysis performed with and without oxidation to determine As(III).

Other colorimetric methods include: As(III) determination with tetraiodomethylene blue iodate (87); oxidation of arsine in $K_3Fe(CN)_6$ solution (88); silver ion reduction with arsine (89); As(V) determination with the O-O-coordination reagent quercetin (90); arsine reaction with methylphenyldimercaptothiopyrone (91); and arsine reaction with $AgSCSN(OC_2H_5)_2$ in pyridine (92).

Other chemical methods include: a ring oven technique after arsenite reaction with potassium thiobicarbonate (93); As(III) by a thin-layer chromatographic technique (94); a column chromatography technique whereby Ag_3As is collected from silver ion reduced with arsine (95); a semi-quantitative technique for AsO_4^{-3} by reaction with KI after removal of nitrite ion (96); AsO_3^{-3} and AsO_4^{-3} detection by ion exchange adsorption followed by reaction with specific reagents (97); adsorption of arsenicals on $Fe(OH)_3$, followed by reduction to arsine, then by co-precipitation from gold chloride ($AuCl_3$) solution (98); and a kinetic method based on As(III) catalysis of the reduction of BrO_3^- in pH 5 solution by I^- (99).

Not an analytical method itself, paper chromatography can be used to separate various arsenic species, each of which can be analyzed separately. One method separates different arsenic-based pesticides from plant tissues and uses the SDDC method of determination (100); another separates arsenate from arsenite and uses the Gutzeit test (101).

Electrochemical methods are becoming more popular for trace analysis. Strictly, only As(III) is measured, As(V) must be converted to As(III) prior to assay. A dead stop titration of As(III) with coulometrically generated iodine was shown to be somewhat more precise than the arsenomolybdate method (102). Direct pulse polarography (103) and single sweep polarography (56, 104, 105, 106) have been used successfully. The most promising technique is stripping voltammetry (57, 58, 107), which has a sensitivity of 0.1 µg/l and differential pulse polarography (58, 59, 60), which has a sensitivity of 0.3 µg/l. One drawback appears to be poor accuracy at high concentrations; a recent EPA report (108) states that differential pulse methods are useful for finished waters and other relatively "clean" surface waters, but are generally too responsive to complex-matrix effects of industrial effluents.

X-ray fluorescence methods require less sample handling, can analyze for several metals simultaneously, and can be non-destructive; but without sample concentration, lack adequate sensitivity for trace environmental analysis. They have been used to determine ppm concentrations in river and sea sediments (61) and organic soils (62). When used to analyze dust collected from air, X-ray fluorescence was able to detect 0.5 µg As (109). Preconcentration by precipitation with diethyldithiocarbamate has been used to analyze ppm levels of arsenic in water (110). A more sensitive approach (63) uses ammonium pyrrolidine dithiocarbamate extraction to concentrate arsenic and is applicable to 0.005-0.5 ppm concentrations.

Gas chromatographic methods are available (14, 64, 65) with sensitivities of 50 ng/l for water samples and 30 ng/g for solids. This method requires the volatilization of arsenic as the trichloride, trifluoride, trimethylsilyl, or triphenyl derivatives. It is time-consuming, and without internal standards may lack the accuracy and reproducibility required.

Methods employing mass spectrometry require a large financial investment in equipment but can simultaneously identify and analyze several elements at low concentrations. Crocker (66) describes analysis of water samples by spark source mass spectrometry. The samples are either evaporated or freeze dried to a residue. Low temperature radio-frequency ashing is used to destroy organics. The ash is mixed with graphite powder (1:1, v/v) and then molded to form a pair of electrodes. At high voltage, only elemental ions are formed,

which are detected on a photoplate after acceleration and separation in the mass spectrometer. Volume size of sample will depend on the solids content. Crocker measured 0.015 mg/l As in 100 ml of river water in one analysis presented, and 0.003 mg/l As in lake water in another analysis, sample size unspecified. The mass spectra of some arsenic compounds have been obtained (67). It is possible that identification and analysis of volatile arsenic species may be accomplished by combined gas chromatography (separation) - mass spectroscopy (identification and analysis). No references were found that use this method.

MAMMALIAN TOXICOLOGY

Human Exposures

The bases for establishing limits for arsenic (see "Existing Standards") are not firmly related to sound experimental data but represent approximations arrived at by the more knowledgeable experts in industrial hygiene and toxicology. Moreover, the use of such limits implicitly assumes that: (a) the most toxic solid compound of arsenic is arsenic trioxide; (b) the most toxic gaseous compound of arsenic is arsine; and (c) any arsenic compound is treated as if it were one of these compounds. The minimum fatal oral dose of arsenic trioxide for man is about 1 mg/kg (1, 111) inhalation of 785 mg/m^3 of arsine in air for 30 minutes is fatal (112).

In the NIOSH Criteria Document (79), a great deal of weight is given to epidemiological studies (113, 114) of a population exposed in an English sheep-dip manufacturing plant from 1910 to 1943 to inorganic arsenicals (sodium arsenite among others). These workers were compared with non-exposed workers in the same village and in a nearby village. Air concentrations in the chemical workers' area could be distinguished from other plant groups and from a control group on the basis of skin pigmentation and warts. The 1975 NIOSH limit (0.002 mg/m^3) was (79) arrived at primarily based upon the foregoing plus the probability that arsenic compounds are also carcinogenic for man; hence it is somewhat arbitrary.

Many reviews of arsenic toxicity and biological activity have appeared (115, 116, 117, 118, 119, 120, 121).

The most common cause of arsenic intoxication in man is the ingestion of inorganic arsenicals in foods, drinking waters, or beverages that have been naturally or accidentally contaminated (111, 122, 123, 124, 125, 126). Signs of intoxication include gastrointestinal disturbances (rice water stools), peripheral neuritis, keratitis, and skin pigmentation. Symptoms include weakness and loss of appetite (127). Chronic exposure to airborne arsenic results in irritation of mucous membranes, dermatitis, pigmentation of the skin, and if severe, perforation of the nasal septum (127).

Urinary excretion of arsenic, although quite variable, has been used over the years as an indication of the degree of exposure. With the development of improved analytical techniques, it is now being realized that the introduced arsenic compounds are converted to other arsenic compounds in the body before being excreted in the urine. It is also being recognized that various organic arsenicals are metabolized at least in part differently from the inorganic arsenicals. Consequently, urinary levels of arsenic are not reliable estimates of inorganic arsenic exposure. The most commonly quoted examples are urinary levels following ingestion of shrimp, lobster, or fish containing relatively high levels of arsenic bound in an as yet unknown fashion (12, 111). Following such ingestion, urinary arsenic levels may increase from a basal level of 0.08 to 0.14 to more than 1 ppm (79). Industrially exposed workers with urinary excretion levels as high as 1 ppm often show signs of arsenic intoxication.

Arsenic levels in human hair for non-exposed populations (from 0.1 to 1 ppm) (128, 129) and of much higher levels in exposed individuals (130, 131) have been used to estimate the intake of arsenic from environmental, accidental, or occupational exposures. The distribution of levels of arsenic in the hair of non-contaminated subjects has been cited as evidence that arsenic is not an essential trace element for man (132).

Carcinogenicity. Arsenic has long been suggested as a cause for many human cancers, but this has been in dispute for over 150 years, since attempts to produce cancer in animal models have generally been unsuccessful.

The occurrence of respiratory and epidermal cancer in men with histories of environmental or industrial exposure to inorganic arsenic compounds has been extensively studied by the Internation Agency for Research on Cancer (116). They concluded that although no animal model system so far tested has shown that arsenic induced cancer unequivocally, causal relationships do exist for man. A later study (133) was made of lung cancer incidence in 36 counties in the United States having smelting and refining operations which release arsenic to the air. In the period 1950-1969, lung cancer deaths in these areas were 17% (men) and 15% (women) above those in areas without such industries. A massive study of inhabitants of an area in Taiwan where well water had a high arsenic content also showed an increased incidence of cancer over a matching population (126). Occupational cancer ascribed to arsenic for 312 cases has been compiled (134). One problem involved with causal studies is the confounding of the alleged arsenic effect with that of other pollutants. However, in 1975, Weisberger has stated that the evidence appears pretty conclusive that arsenic can be a human carcinogen (135). While reported arsenic-induced tumors appear at point

of contact -- that is respiratory and/or skin, the proximal carcinogen is still unknown. In both human and animal urine, the predominant form of arsenic is organically bound, presumably methylarsonic acid, even though exposure is primarily to inorganic arsenic. Thus some intermediate metabolite or the final urinary product could be the proximal carcinogen.

Experimental Animals

Single dose oral LD_{50}'s for inorganic compounds of arsenic are given as 45 mg/kg in rats for arsenic trioxide (1); 20-50 mg/kg in rats for sodium arsenite, 80-120 for sodium arsenate, 1200-1600 for sodium methylarsenate and 1200-1600 for sodium cacodylate (12); 298 for calcium arsenate in rats (136). The oral LD_{50} of arsenic trioxide has been estimated at 20-39 mg/kg for guinea pigs, 14-30 for rabbits and 30-70 for dogs (137). Gastrointestinal irritation is the predominant sign of toxicity.

Repeated dose toxicity studies in animals have been conducted primarily in attempts to develop an animal model system to detect the carcinogenic activity of arsenic or to elucidate storage and excretion phenomena. Not much pathology was seen in these studies until dose levels producing some reduction in weight gain were reached. At such levels, approximating 125 ppm sodium arsenate or arsenite in the diet, enlargement of the common bile duct in rats and changes in the hemograms in rats and dogs were observed (138). Although several dogs died, the only microscopic lesion seen was deposition of some abnormal pigments in the liver. By the inhalation route, rats are said to have exhibited morphological, behavioral, and neurological changes at 4.9 but not at 1.3 $\mu g/m^3$ constant exposure for three months (139, 140).

Other investigators have reported some biochemical lesions in animal studies such as increased serum cholesterol, reduced cholinesterase activity, and increased blood pyruvate. These observations remain to be confirmed and their significance, if any, to be defined (79).

Peoples, in 1964, investigated some aspects of the toxicity of arsenic in cattle along with rats, guinea pigs, rabbits, and hamsters (141). He fed 0.05, 0.25 and 1.25 mg/kg/day of arsenic acid for eight weeks to lactating Holstein cows. No arsenic above pretreatment levels appeared in the milk; the analytical method used appears to have a 0.05 ppm arsenic detection level. No clinical signs and no histological changes at autopsy were seen. Tissue levels revealed little or no storage, with 2 ppm arsenic in the liver of a high dose-level animal being the highest reported. Urinary excretion of arsenic accounted for 54 to 98 percent of the daily dose. By contrast, a dose of 1.25 mg/kg/day in rats produced tissue levels of 22 ppm in liver, 18 in kidney, 38 in spleen, and 150 in blood. The rabbit gave a storage level pattern most nearly like that of cows, while elevated storage occurred in the liver, heart, and spleen

tissues of guinea pigs and hamsters, although not as high as that noted with rats. From this study, Peoples has questioned the use of the rat for the purpose of extrapolating arsenic toxic effects to other animals (12, 141).

Sodium arsenate caused teratogenic effects in hamsters when applied by intravenous injection (142). Schroeder and Mitchener (143) failed to find teratogenic effects in mice drinking water containing 5 ppm arsenic in the form of arsenite but did observe reduced litter size through the third generation. Hood and Bishop (144) found decreased fetal weights, increased fetal resorptions and increased fetal anomalies in pregnant mice injected intraperitoneally with 45 mg/kg sodium arsenate on one day of the sixth through eleventh day of gestation, but not with 25 mg/kg. Effects of sodium arsenite on fetal development have also been studied by Hood (145) with a similar injection methodology. A noticeable increase in fetal deaths and skeletal anomalies was noted with 10 or 12 mg/kg arsenite. Malformations in the golden hamster following the injection of 15-25 mg/kg sodium arsenate intravenously on day 8 of gestation have been noted (146); in the rat following 20-30 mg/kg intraperitoneally on days 8, 9 or 10 (147); in the mouse at 10 and 40 mg/kg orally on days 9, 10 and 11 (148).

Metabolic studies in animals have shown that the highest concentrations of arsenic are found in the kidneys, liver, spleen, and lung (141, 149, 150, 151). The digestive process involves arsenic-compound conversions; arsenite-arsenate equilibrium has been observed in the kidney of the dog (152). Moreover, digestion of arsenate by dogs and cows is followed by the appearance of methylarsenate in the urine (153). The bile represents a major route of excretion of both arsenate and arsenite to the intestine. However, most of the arsenic is absorbed from the intestine (154, 155) and eventually excreted via the urine (152).

Mice dosed subcutaneously or fed in drinking water sodium arsenite had two- to ninefold mortality rates of non-dosed controls when inoculated with selected viruses (156). Interference with interferon formation and action has been suggested as the reason for this increased susceptibility (157).

Chromosome damage has been noted in cell culture; in some cases, human cell culture at concentrations of the order of 0.1 to 1 µg/ml of $Na_2HAsO_4 \cdot 7H_2O$ (158), 10^{-6} molar arsenite (159) and 10^{-8} molar arsenate or arsenite (160).

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Arsenic compounds, despite their notoriety as poisons, are ubiquitous in the environment. Several reviews discuss their occurrence; an overall review by Woolson (161), a soil review by Walsh and Keeney (162), and a water review by Ferguson and Gavis (163). At best, naturally occurring arsenic has to be accommodated by public health, plants and wildlife. At worst, it presents a problem in localized areas, such as Antofagasta, Chile, where untreated river water used for drinking purposes contained approximately 0.5 ppm As (123), and parts of Taiwan, where untreated artesian well water had 0.1 ppm or higher As (126). However, man's additions to the environment, from smelting of ores or coal burning, waste discharges from pesticide and feed manufacture*, and excessive or repeated use of arsenic pesticides, have caused the major problems arsenic has posed. For example, man's activities are estimated to account for 75 percent of the arsenic transported to the oceans by rivers (163).

Research into environmental arsenic shows that a rather diverse transport cycle exists, paralleling that of mercury. In aerated, unsaturated soil, As(III) oxidizes slowly to As(V). This is aided in part by microorganisms (164). In waterlogged soils, the reverse occurs (165), and microorganisms may also be involved (162). Under such conditions, arsine gas may be formed (162, 165), although its detection has not been well established. Arsenic may be "fixed" in soils due to ferric ion or sulfide. In soil and water, organisms, perhaps as a detoxifying mechanism, convert As(V) to methylarsonic acid or dimethylarsinic acid (7, 163). These compounds can, in turn, be biologically converted to the toxic gases dimethylarsine or trimethylarsine (163). Fortunately, these gases are rapidly oxidized in air to As_2O_3, which either settles to earth or is washed out by precipitation.

Soils. Persistence---Applied arsenic displays great persistence in some soils. In one study (83), arsenic was measured in the top six inches of a sandy loam soil from an orchard where annual applications of lead arsenate were made over a 32 year period (ending four years previous to the assay). About 30 percent of the applied arsenic was still present**. A similar study (166) involved arsenic measurements

*Certain organic arsenic compounds are used in poultry and cattle feeds.
**These calculations were based on the assumption of uniform arsenic content in the soil layer and a soil density of 150^3lb/ft or 2.5 g/cc.

on Berwick sandy loam from 1954-1958 after $PbHAsO_4$ was applied at a 419 lb/acre* rate annually from 1949-1953. In annual measurements, the arsenic content ranged from 126-157 ppm with no pattern of depletion. About 90 percent of the arsenic applied was retained. The authors concluded that arsenate was more persistent in the soil than chlordane (166).

Distribution. Applied arsenic is retained in surface layers. In one study (167), plots of Matapeake silt loam were dosed with 2690 kg/ha $NaAsO_2$. Fourteen years later, arsenate assays ranged from 318 ppm in the 0-8 cm deep soil layer to 42 ppm in the 38-46 cm deep layer. In another study (168), various doses of $NaAsO_2$ were applied to Plainfield sand. Three years later, the control plot (no $NaAsO_2$ applied) assayed 3.6 ppm As in the 0-23 cm layer, while depths to 83 cm assayed from 1.2 to 1.6 ppm As. In plots dosed with 180 kg/ha, the 0-23 cm layer had 45 ppm As, the 23-38 cm layer had 4.8 ppm As, and lower layers were virtually unchanged from the control.

Adsorption. Adsorption data for sodium arsenite-soil equilibria were reported in 1966 by Sundd and Bansal (169). Their data was in awkward form, and has been recalculated to yield the information in Table H-3. The As soil content in equilibrium with 1 ppm As in water (a fairly high water content) has been computed.

TABLE H-3. Adsorption Isotherm Parameters of $NaAsO_2$ in Selected Indian Soils (169).

Soil	α†	β†	C_s for C_w=0.001 (1ppm)
Bhopal Black Cotton	3.18	.437	.155 (155 ppm)
Delhi Clay Loam	1.30	.606	.020 (20 ppm)
Delhi Sandy Loam	1.23	.644	.014 (14 ppm)
Laterite	6.20	.464	.251 (251 ppm)

†α and β are in units to fit $C_s = \alpha C_w^{\beta}$, where C_s is in mg As/g soil and C_w is in mg As/cc water.

They noted that a high ferric ion content or a large exchangeable calcium and magnesium ion content in soil strongly fixed arsenic. A similar

*Typical application rates are about 10-50 lb/acre or 8.9-45 kg/ha.

study was reported by Jacobs et al. (170) for three selected Wisconsin soils equilibrated with Na_2HAsO_4 solution. From their graphical results, Langmuir isotherms have been computed (see Table H-4).

TABLE H-4. Adsorption Isotherm Parameters of Na_2HAsO_4 in Selected Wisconsin Soils (170)

Soil	K*	b*	C_s for C_w=1 (1 ppm)
Superior clay loam	28.1	0.0146	27.7 ppm
Waupun silty clay loam	17.7	0.0152	17.4 ppm
Plainfield sand	7.7	0.0124	7.6 ppm

*K and b are in units to fit $C_s=KC_w/(1+bC_w)$, where C_s is micrograms of As/g soil and C_w is microgram As/ml water.

Effect of Soil Chemicals. A number of articles by Woolson and co-workers qualitatively explain arsenic persistence as a function of soil components. In one study (16), 58 arsenic-contaminated soils and corresponding samples of non-contaminated soils were used for corn growth tests. The soils were analyzed for the water-soluble, iron-, aluminum- and calcium-arsenic fractions. Moreover, the soils were analyzed for "exchangeable" calcium (extractable in 0.5N NaOAc solution), "reactive" iron (extractable in oxalate solution), and "reactive" aluminum (extractable in 1 N NH_4OH solution). An analysis of the results of corn growth reduction, arsenic and cation assays showed the following: As accumulates in soils with high reactive iron content ($FeAsO_4$ is the least soluble salt listed in Table H-1); if reactive iron content in soil is low, arsenic may accumulate in soil with high reactive aluminum or exchangeable calcium content; if none of these exchangeable or reactive cations is present, arsenic in soil is more phytotoxic and easier to leach. In a 1970 article by Jacobs et al. (170), a similar trend was observed for ferric/aluminum ion absorption of arsenic in Wisconsin soils treated with Na_2HAsO_4.

In 1973, Woolson et al. (5) reported a study of the uptake of arsenic with time in the four above-cited fractions of soils in Lakeland sandy loam and Hagerstown silty clay loam after different application levels of Na_2HAsO_4 (a water-soluble arsenate). The Hagerstown soil attained equilibrium in about 4 weeks. At the 100 ppm As application level under equilibrium conditions in that soil, about 55 percent of the arsenic was bound with iron and about 30 percent of the arsenic was bound with aluminum.

The Lakeland soil took longer to equilibrate. At the 100 ppm As application level under equilibrium conditions, comparable arsenic fractions in the iron and aluminum fractions were 0.22 and 0.55 respectively. When equilibrium was attained, about 12 percent of the arsenic in the Lakeland soil was still water-soluble; virtually none of the arsenic in the Hagerstown soil was water-soluble.

Effect of Soil Microorganisms. Bautista and Alexander (171) isolated two soil organisms, Pichia guillermondii and Micrococcus sp., capable of reducing 10^{-3}M sodium arsenate to arsenite. This property was possessed by growing cells, resting cells, and the soluble fraction of cell-free extracts. Organisms capable of producing arsenite in the presence of sodium arsenate, 150 ppm as arsenic, numbered 6.7×10^4 fungi and 4.6×10^7 bacteria per gram in a Lucas silty clay loam (171).

In other studies, Quastel and Scholefield (164) showed that microorganisms in aerated soil convert arsenite to arsenate, Epps and Sturgis (165) showed that arsenate was reduced in water-logged soils, but neither study actually isolated the causative organism(s).

Water. With respect to arsenic transport in water, Ferguson and Gavis (163) note that from past surveys, 7-21 percent of rivers tested had As of more than 10 ppb while only 0.5 percent of drinking waters did. Apparently, water treatment processes inadvertently reduce As concentrations. The most likely reason, although not rigorously established, is through adsorbtion on sediment.

Sediment Removal. Other studies which point to high As removal by sediment are by Wilder (172) and Seydel (173). In the former, As concentrations reached 1100 µg/l in Sugar Creek, 17 miles downstream of the Irving Creek sewage treatment plant of Charlotte, NC. The source was speculated to be from wastes of an arsanilic acid plant. At the time sewage treatment plant effluent was sampled, 115-260 µg/l (ppb) As was noted. However, suspended solids in the effluent (11-75 mg/l) contained 24,000 to 500,000 µg/kg (24-500 ppm) arsenic. The stream-bed sediment below the plant built up to 35 ppm 6.4 miles below the plant and decreased to 8.1 ppm 17 miles below the plant. In the latter study, water and sediment samples were taken of various locations around Lake Michigan. Water assays ranged from 0.5 to 2.4 ppb, while sediments ranged from 7.2 to 28.8 ppm.

Aquatic Microbiological Activity. Wastewater organisms can convert arsenic between arsenate and arsenite. The equilibrium achieved is highly dependent on oxygen tension (171, 174, 175, 176). As an example, Heimbrook (175), isolated from river water, sewage and activated sludge strains of Pseudomonas, Escherichia, Enterobacter, Achromobacter and Alkaligenes that were active in reduction of arsenate or oxidation of arsenite.

Broth filtrates of the organisms and autoclaved suspensions were inactive. Reduction of arsenate by washed cells of Pseudomonas fluorescens required an energy source and was inhibited by phosphate. Oxidation of arsenite by A. fecalis was not accompanied by phosphate inhibition. Activated sludge was the most efficient oxidizer of arsenite.

Braman and Foreback (7) have detected the methylated arsenicals methylarsonic acid and dimethylarsinic acid in rivers, lakes and saline waters of the Tampa, FL area. Apparently, these compounds arise from fungal, bacterial or animal digestion of arsenic, although they are also used as pesticides. Cox and Alexander (177) isolated from raw sewage three fungal isolates that produce trimethylarsine from monomethylarsonic acid or dimethylarsinic acid at pH 5, 6 or 7. One, Candida humicola, yielded trimethylarsine from arsenate or arsenite.

Johnson and Pilson (178) have estimated the rate of oxidation of added As(III) to As(V) in seawater at 0.023 µmol/l-year at 4°C, based on experimental work. At such a rate, naturally occurring arsenite in seawater would be depleted; apparently biological reduction maintains a balance between the two valance states. The oxidation is believed in part due to photochemical reactions, in that reaction rates were five to ten times higher in sunlight than in laboratory light or darkness.

Air. Arsenic in air ranges from below detectable limits to 0.75 µg/m^3, with an average of about 0.02 µg/m^3 (161). A major source of this arsenic is from the burning of coal, which may have up to 2000 ppm As. One calculation of the amount of arsenic in air generated from the amount of coal burned in Yew York City agreed exactly with the average As content in the air measured there, 0.03 µg/m^3 (161). Arsenic is washed from air by rain or snowfall (161, 179). The major source of arsenic found in rain and snow samples in Japan (arsenic contents from 0.01 to 13.9 µg/l with an average of 1.6 µg/l) has been theorized as from industrial sources, with windblown particulate soil as a secondary source (179).

Background Concentrations. Woolson (161) cites arsenic contents in uncontaminated soils as from 0.1 ppm to 40 ppm, with an average content of 5-6 ppm. Other contents are presented by Walsh and Keeney (162): Colorado soils, 1.3 to 2.3 ppm; Maryland soils, 19-41 ppm; and 4 to 80 ppm in Washington State soils. Some of the higher contents may be a consequence of arsenic-treated soils from nearby areas being deposited on the area tested by air transport. Up to 8000 ppm As has been reported in soils overlaying sulfide ore deposits (161). Naturally occurring arsenic in soils appears to be uniformly distributed with depth (180).

Surface water arsenic contents are generally below 10 ppb; as noted previously, surveys indicated that 7 to 21 percent of waters assayed were above this value (163). Groundwater arsenic assays typically range from below detectable amount to 1700 ppb (161). Some extreme situations of As contents in river and well waters have been cited, these are probably associated with contact with arsenic-rich ores.

Animals

Mammals: No information was found on the effect of arsenic in the environment to free-living mammals.

Birds: Birds are able to withstand up to 4800 mg/l lead arsenate in their drinking water for at least 60 days (15). Chickens are killed with doses of 1.3 to 56.7 g/day lead arsenate; 324 mg of arsenic trioxide killed chickens within 24 hours. Arsanilic acid ($NH_2C_6H_4AsO(OH_2)$) levels in diets of turkey poults caused weight loss at concentrations above 0.02 percent (in total diet) and mortality at 0.08 percent (181).

Amphibians: The following compounds in the cited concentrations in solution caused 50 percent mortality in tadpoles: 195 mg/l arsenic pentoxide or 910 mg/l arsenic trioxide within 30 minutes; 310 mg/l sodium arsenate or 130 mg/l sodium arsenite within 600 minutes (182).

Fish: Some fish are extremely sensitive, e.g., pike 1.1 mg/l, bluegills 4 mg/l; others somewhat less, e.g., minnows 11.6 mg/l (15). Low concentrations of arsenous acid (1 ppm) may function as an emetic when ingested by walleyes (183). For a summary of known values for toxic concentrations, see Table H-5.

TABLE H-5. Toxic and Tolerable Concentrations of Arsenic to Certain Higher Aquatic Organisms and Time of Exposure (15)

Type of fish	Toxic concentration of arsenic (mg/l)	Time of exposure	Tolerable concentration of arsenic (mg/l)	Time of exposure
Bass	7.6	10 days	6.0	232h
Bleak	2.2	3 days	1.1-1.6	11 days
Carps	3.1	4-6 days	2.2	13 days
Crab	4.3	11 days	3.1	90 days
Eels	3.1	3 days	2.2	13 days
Minnows	11.6	36 days	13.0	1 h
Pike, perch	1.1-2.2	2 days	0.7-1.1	48 days
Trout	---	----	7.6	30 days

Invertebrates: Aquatic insects appear sensitive to arsenic in water. In the above cited reference (15), 3-14 mg/l As is toxic to mayflies, 10-20 mg/l As is toxic to dragonflies, and Daphnia are killed by 4.3 to 7.5 mg/l As. On the other hand, flatworms (Polycelis) can tolerate up to 361 mg/l (15).

Microorganisms: Sodium arsenate solution with an arsenic content of 290 mg/l is toxic to E. coli, but other bacteria can withstand 10^4 mg/l sodium arsenate (15). Yeast fermentation ceased in 300 mg/l arsenate (15). Sodium arsenate concentrations of 250 to 500 ppm in soil were not harmful to soil microorganisms (184).

Bacteria occurring in river water, sewage and activated sludge exhibit few toxic effects at 5 mg As/l (175). Some sewage organisms such as some Bacillus, Streptococcus or Pseudomonas strains are tolerant of 1000 mg/l of either arsenate or arsenite (176).

The yeast Rhodotorula rubra grows at extremely low phosphate concentrations. The highly effective phosphate transport system does not discriminate against arsenate, so that toxicity occurs at arsenic concentrations down to 1 to 10 nM (185). This is partially reversed by higher phosphate concentration (0.7 to 1.2 μm) which competitively inhibits arsenate uptake (185).

Greaves (186) studied microbial attack on wooden stakes, impregnated with a commercial mixture of arsenic pentoxide, sodium arsenate, potassium dichromate and copper sulfate, driven into soil in a tropical forest region. The arsenic level in the treated wood was as high as 1.9 kg/m^3. The preservative retarded decay but had little effect on the variety of organisms, especially species of Penicillium, Streptomyces, and Bacillus that invaded the wood. When these species were tested for resistance to a 3 percent solution of the same mixture, about 0.8 percent arsenate, most fungal isolates showed son resistance, while most bacteria and actinomycetes showed little. Some fungi species, Fusarium and Chaetomium, exhibited detoxification of the mixture by precipitation with extracellular pigments.

Genera such as Bacillus, Streptococcus, Pseudomonas, Alcaligenes, Achromobacter, Escherichia, Herellea, Enterobacter and Proteus are able to reduce arsenate to arsenite under anaerobic conditions (175); many of these are arsenic-tolerant species.

Plants

Phytotoxicity: The phytotoxicity of arsenic depends upon the availability of arsenic in soil and is related to initial arsenic contamination, soil

pH, soil content of iron, aluminum, calcium, and phosphorus (5, 187, 188, 189), and to genetic differences in plant species and different plant root absorption zone depths (82, 190, 191). Phytotoxic symptoms from arsenic include wilting, necrotic leaf tissues, altered fat metabolism, disorganization of root systems, and retardation of germination and growth (15, 187, 192, 193, 194). In addition, the chemical form in which arsenic is applied to soil influences phytotoxicity more than the total amount of arsenic present in the soil (15, 16, 84, 165, 195). In tomato, sudan grass, and beans, arsenite is a more toxic form than arsenate (15). Arsenites inhibit growth in rice plants (196) and germination of lettuce (193) more than do arsenates. Applied chlorate and borate salts may enhance arsenic phytotoxicity (197).

The availability of arsenic fractions in soils has been correlated with plant injury (see Table H-6).

TABLE H-6. Total Dry Weight of Selected Crops vs. Available Arsenic* (189).

Crop	Regression Eqtn**	r
Green beans	y = 77 - 34 Log x	0.89
Lima beans	y = 107 - 55 Log x	0.83
Spinach	y = 88 - 3 Log x	0.91
Cabbage	y = 114 - 38 Log x	0.80
Tomato	y = 109 - 42 Log x	0.87
Radish	y = 96 - 36 Log x	0.81

*Available arsenic is that which is extractable from soil with 0.05 N HCl and 0.025N H_2SO_4 solution.

** y = percent of control-plant weight, x = available soil As, ppm. Arsenic added as $Na_2HAsO_4 \cdot 7H_2O$.

Table H-6 shows that plant species differ in their susceptibility to arsenic. Green beans have a 50 percent growth reduction with 6.2 ppm available arsenic in soil while a 50 percent growth reduction in cabbage requires 48.3 ppm available arsenic in soil. Arsenate in soil becomes injurious to corn plants at about 10 ppm with water-soluble compounds (i.e., Na_2HAsO_4) being the most toxic and Fe-As (i.e., $Fe(H_2AsO_4)_3$) the least toxic (84). C. fragilis, an alga, is able to grow in $NaAsO_2$ and $NaHAsO_4$ solutions because the arsenic is made insoluble in the thalli (9). The grass Andropogon scoparius Michx. has an evolved arsenic tolerance, with some individuals of this species able to grow in soil containing 41,200 ppm arsenic (198).

Arsenic phytotoxicity has also been observed during growth of red pine trees, hemlock trees, white spruce seedlings and blueberry bushes (199, 200, 201, 202).

Bioaccumulation: Bioaccumulation of arsenic in plant tissue depends upon the same factors which influence phytotoxicity of arsenic. Regression equations for the arsenic uptake as related to the available arsenic in soil have been calculated for some plants (see Table H-7). Correlation coefficients generally indicate a close fit between these variables. Studies on arsenic distribution in the aerial parts of bean plants indicate that highest concentrations are generally found in leaves and lowest concentrations in fruit (190). Woolson (189) reports that plant roots have higher arsenic residue accumulations than do plant tops. Radishes, a root crop, contained 76 ppm arsenic residue (dry weight basis) when 19 ppm was available in soil. At this level, radish growth was reduced 50 percent (189). Arsenic accumulation has also been measured in corn, beans, carrots, Swiss chard, and turnips grown on soils from old orchards which had been sprayed with lead arsenate (203). The addition of phosphate to soil can increase the accumulation of arsenic in plant tissues (5). Applications of ferric sulfate and aluminum sulfate did not increase arsenic phytotoxicity in peas or potatoes nor arsenic accumulations in potatoes (168). Arsenic contents of potato peelings varied with available soil arsenic, however the arsenic content of potato flesh did not exceed 0.6 ppm (204).

TABLE H-7. Uptake of Arsenic in Whole Dry Plants and Edible Dry-Weight Portions (189)

Crop	Regression Equation* and (Correlation Coefficient) Whole Dry Plant	Edible Dry-Weight Part
Green beans	y = 0.4 + 4.2Log x (.93)	y = 0.43 + 4.83Log x (.44)
Lima beans	y = 0.5 + 1.2Log x (.49)	y =-0.15 + 1.12Log x (.75)
Spinach	Log y =-0.13 + 1.1Log x (.90)	See whole dry plant
Cabbage	y = 0.4 + 1.8Log x (.77)	y = 0.77 + 0.39Log x (.50)
Tomato	y =-0.1 + 3.3Log x (.80)	y = 0.54 + 0.12Log x (.29)
Radish	Log y =-0.15 + 1.4Log x (.88)	Log y =-0.29 + 1.7Log x (.90)

* y = ppm Arsenic in portion of plant cited, x = available soil arsenic, ppm. Arsenic added as $Na_2HAsO_4 \cdot 7H_2O$.

In a review article, Nash (187) summarized arsenic accumulations in plants grown in soils containing various arsenic levels: residues of up to 76 ppm in barley tops (308 ppm As in soil); 0.014 ppm in rice grain

(338 ppm As in soil); 0.040 ppm in carrot roots (157 ppm As in soil); and 0.52 ppm in bean seeds (127 ppm As in soil).

Fresh water aquatic plants have concentration factors ranging from 100 to 20,000 (205). Aquatic marine plants contain 71,000 times as much arsenic as is present in ambient sea water (206).

Food Chains

Most higher plants apparently do not strongly concentrate arsenic from soil. However, accumulation does occur in marine lower plants and animals, and to a lesser extent in their fresh-water counterparts. Woolson cites bioaccumulation ratios (content of arsenic in organisms to that in water) in his review (206), some of which are presented in Table H-8. The arsenic compound involved is an important factor in the observed ratios.

TABLE H-8. Bioaccumulation Ratios of Arsenic in Selected Organisms in Water (206)

Organism	Water	Ratio	Remark
Algae	Fresh	3 - 17	Arsenate source
Snails	Fresh	2 - 21	Arsenate source
Catfish	Fresh	<1	Arsenate source
Crayfish	Fresh	<1 - 16	Arsenate source
Algae	Fresh	163 - 27,000	$((CH_3)_2AsO(OH))$ source
Snails	Fresh	4 - 1,000	$((CH_3)_2AsO(OH))$ source
Catfish	Fresh	2 - 275	$((CH_3)_2AsO(OH))$ source
Crayfish	Fresh	3 - 14	$((CH_3)_2AsO(OH))$ source
Seaweed	Sea	30,000 - 71,000	2 µg/l As assumed
Algae	Sea	50 - 47,000	2 µg/l As assumed
Crustacea and shellfish	Sea	9 - 1,550	2 µg/l As assumed
Assorted fish	Sea	38 - 1,135	2 µg/l As assumed
Assorted fish	Fresh	3 - 30	10 µg/l As assumed

Seydel (173) has studied the Lake Michigan environment. Lake water contains from 0.5 to 2.4 ppb As, while sediments contain 7.2 to 28.8 ppm As, benthos from 4.7 to 8.8 ppm As, phytoplankton from 4.2 to 9.6 ppm As,

and zooplankton from 4.1 to 7.9 ppm As. Benthic organisms ingest some sediment which contributes to their As content. Phytoplankton take As from water and may either act as adsorption sites for As, or As(V) may be taken up in lieu of P(V). Accumulation of As in phytoplankton and zooplankton may result from more rapid uptake than their metabolic and excretory processes can handle.

EXISTING STANDARDS

Various standards for arsenic are presented in Table H-9. Some of the rationale (or lack of it) for these values have been discussed in the "Human Toxicology" section. A joint FAO/WHO maximum acceptable intake of 0.05 mg/kg-day for humans has been tentatively established (207).

TABLE H-9. Recommended or Prescribed Limits for Arsenic

Kind of Compound	Exposure	Agency	Limit	Ref
Inorganic Arsenic*	Occupational	ACGIH	0.5 mg/m^3 air	112
Inorganic Arsenic	Occupational	NIOSH	0.002 mg/m^3 air	79
Arsenic	Occupational	Various foreign	0.15-0.5 mg/m^3 air	79
None specified	Drinking water	EPA	0.05 mg/l	70
Arsenic as As_2O_3	Food Residue	FDA	3.5 ppm**	208
Arsenic	Cottonseed Products (food)	FDA	0.2 ppm***	209

*TLV for calcium arsenate (as compound) 1.0 mg/m^3 and for lead arsenate 0.15 mg/m^3 (112).
**Tolerance as set for magnesium, sodium, copper and calcium arsenate in or on raw agricultural commodities.
***Based on background levels; no added arsenic compounds allowed.

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APPENDIX I

MERCURY AND MERCURY SALTS

COMPOUNDS CONSIDERED

Hg, $HgCl_2$, HgS, $Hg(OOCCH_3)_2$

PHYSICAL AND CHEMICAL PROPERTIES

CAS Reg. No. and Toxic Substances List

Mercury, 7439976; OV45500
Mercury Chloride (II), 7487947; OV91000
Mercuric Sulfide, 1344-48-5; No TSL
Mercuric Acetate, 160027; A185750

Mercury is a unique metal, a silvery liquid at room temperature; hence it is commonly known as "quicksilver". The average proportion of mercury in the earth's crust is about 5×10^{-5}% (1), but most of this is concentrated in deposits. The natural isotopes of mercury are 196, 198, 199, 200, 201, 202, and 204; the mean atomic weight of mercury from these isotopes is 200.59 (1).

A review of mercury and mercuric compound use appears in a 1972 monograph by D'Itri (2). Elemental mercury has been known to man from prehistoric times and has been purified from ores (mostly cinnabar, i.e., HgS) since the Roman Empire period. It is widely used in scientific and laboratory equipment, in electronics, in the paint and paper industry, in the production of fungicides and bactericides, in the preparation of amalgams, and as a wood preservative, fungicide, and catalyst in the production of vinyl chloride and lewisite. Mercuric acetate has no known commerical uses. Physical properties of these materials appear in Table I-1.

Mercuric chloride hydrolyzes rapidly in water. Lilich, et al., in 1956 (5) determined the pH of aqueous solutions of the salt. A solution of 0.0115 *M* $HgCl_2$ had a pH of 4.26; a solution of 0.104 *M* $HgCl_2$ had a pH of 3.65. The aqueous chemistry of mercuric chloride is complicated by the formation of charged complexes in addition to undissociated $HgCl_2$ (6).

Inorganic mercury salts dispersed into a saline environment in contact with atmospheric oxygen can form a variety of soluble and insoluble mercury compounds. The nature of the mercury compounds formed is a function of the oxygen concentration (oxidation-reduction potential), chloride concentration and solution pH. Equilibrium concentrations of each chemical species can be calculated from available thermodynamic data, which are summarized in Tables I-2 and I-3 (7).

TABLE I-1. Selected Physical Properties of Mercury, Mercuric Chloride, Mercuric Acetate, and Mercuric Sulfide (1, 3, 4)

	Mercury (Hg)	Mercuric Chloride ($HgCl_2$)	Mercuric Acetate ($Hg(OAc)_2$	Mercuric Sulfide (HgS)
Melting point, °C	-38.8	276	decomposes	1450 (120 atm)
Boiling point, °C	356.6	302	---	583.5
Density at 25°C, g/cc	13.53	5.44	3.27	8.10, 7.73*
Vapor pressure at 20°C, mm Hg	1.2×10^{-3}	---	---	---
Temp. in °C for given vapor pressure, in mm Hg:				
1	126	---	---	---
10	184	180	---	---
100	261	235	---	---
Solubility, g/100 g water at given temp. in °C	63×10^{-7}(25) 261×10^{-7}(80)	6.6 (20) 58 (100)	25(10) 100(100)	1×10^{-6}(18)

* Respective densities for two crystalline forms, cinnabar and metacinnabar

TABLE I-2. Selected Electrode Potentials

Half-reaction	Reduction potential, volts
$O_2(g) + 2H^+ + 2e^- \rightleftarrows H_2O_2$	+ 0.682
$Hg_2^{++} + 2e^- \rightleftarrows 2Hg(l)$	+ 0.789
$Hg^{++} + e^- \rightleftarrows \frac{1}{2} Hg_2^{++}$	+ 0.920
$H_2O_2 + 2H^+ + 2e^- \rightleftarrows 2H_2O$	+ 1.77

TABLE I-3. Selected Equilibrium Data*

$$[Hg_2^{++}][Cl^-]^2 = 1.32 \times 10^{-17},$$

$$[Hg_2^{++}] = 130[Hg^{++}],$$

$$[Hg^{++}][Cl^-]^2 = 10^{-13.79},$$

$$[HgCl^+] = 5.5 \times 10^6[Hg^{++}][Cl^-],$$

$$[HgCl_2] = 1.65 \times 10^{13}[Hg^{++}][Cl^-]^2,$$

$$[HgCl_3^-] = 1.17 \times 10^{14}[Hg^{++}][Cl^-]^3,$$

$$[HgCl_4^=] = 1.17 \times 10^{15}[Hg^{++}][Cl^-]^4,$$

$$[Hg_2Cl^+] = 20[Hg_2^{++}][Cl^-],$$

$$[H^+][HgOH^+] = 2.0 \times 10^{-4}[Hg^{++}],$$

$$[H^+]^2[Hg(OH)_2] = 5.0 \times 10^{-7}[Hg^{++}],$$

$$[H^+][Hg_2OH^+] = 1.0 \times 10^{-5}[Hg_2^{++}],$$

$$[H^+][OH^-] = 1.0 \times 10^{-14}.$$

* Concentration units: moles/liter.

For the equilibrium system including the components Hg, Cl^-, H_2O, and O_2 there are four possible insoluble mercury compounds: Hg(l), $Hg_2Cl_2(s)$, $HgCl_2(s)$, and HgO. There are ten possible soluble mercury species: $[Hg_2^{++}]$, $[Hg_2Cl^+]$, $[Hg^{++}]$, $[HgCl^+]$, $[HgCl_2]$, $[HgCl_3^-]$, $[HgCl_4^=]$, $[Hg_2OH^+]$, $[HgOH^+]$, and $[Hg(OH)_2]$. In the presence of atmospheric oxygen at chloride concentrations above 1 mg/l and in the pH range 5 to 12, the predominant mercury species are shown to be $HgCl_2(s)$, HgO(s), $[HgCl_2]$, and $[HgCl_4^=]$.

The equilibrium data for predominant mercury species are summarized in Figure I-1, where chloride concentrations have been converted to mg/l. The $HgO/HgCl_2$ solid phase equilibrium involves the equilibrium:

$$HgO(s) + [H_2O] + 2[Cl^-] = HgCl_2(s) + 2[OH^-]$$

with the corresponding equilibrium constant

$$K = [OH^-]^2/[Cl^-]^2 = 10^{-11.707}$$

and the corresponding equilibrium line defined by

$$pH = 8.146 - \log [Cl^-],$$

where $[Cl^-]$ is expressed as moles/liter. To the right of the phase equilibrium line of Figure I-1 the stable mercury compound is HgO(s) and to the left of the line the stable mercury compound is $HgCl_2(s)$.

Three equilibrium lines are shown for various total dissolved mercury concentrations. Thus the line marked 100 ppm defines the pH values and chloride concentrations at which the total dissolved mercury concentration is 100 mg/l of mercury (4.985×10^{-4} moles/l). Above pH 8.6, where the predominant soluble species is $[HgCl_4^=]$, the relevant reaction is

$$HgO(s) + 4[Cl^-] + [H_2O] = [HgCl_4^=] + 2[OH^-],$$

with the corresponding equilibrium constant

$$K = [HgCl_4^=]\,[OH^-]^2/[Cl^-]^4 = 10^{-10.428},$$

and the corresponding equilibrium line

$$pH = 10.437 - 2 \log [Cl^-].$$

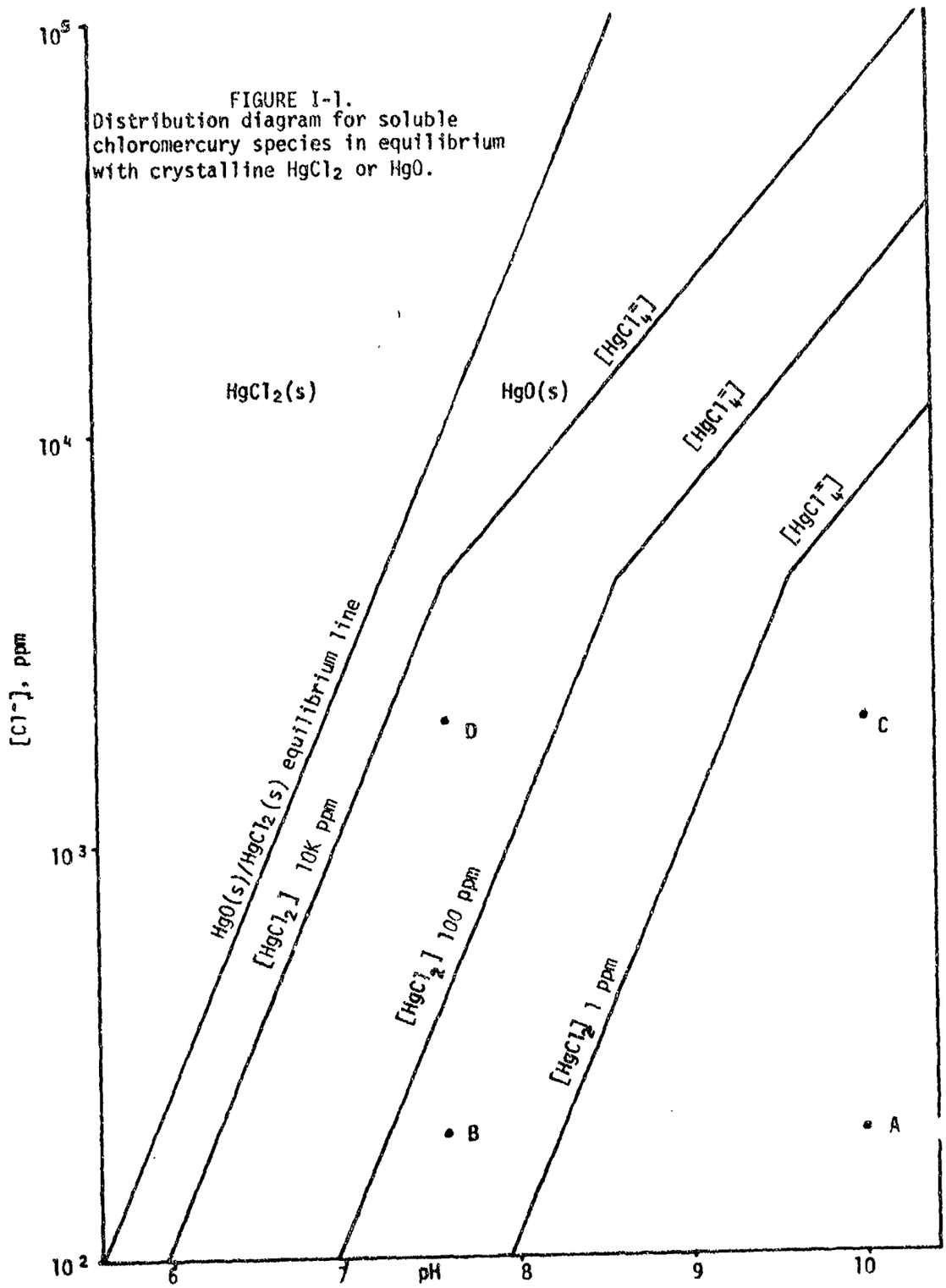
Here $[Cl^-]$ is expressed in moles/liter. Below pH 8.6 the predominant soluble species is $[HgCl_2]$, where the relevant reaction is

$$HgO(s) + 2[Cl^-] + [H_2O] = [HgCl_2] + 2[OH^-],$$

the corresponding equilibrium constant is

$$K = [HgCl_2][OH^-]^2/[Cl^-]^2 = 10^{-12.280},$$

FIGURE I-1.
Distribution diagram for soluble chloromercury species in equilibrium with crystalline $HgCl_2$ or HgO.



and the corresponding equilibrium line

$$pH = 9.511 - \log [Cl^-].$$

Here also, $[Cl^-]$ is expressed in moles/liter. Similar calculations yield the equilibrium lines for 1 ppm and 10,000 ppm total dissolved mercury.

With Figure I-1 it is possible to quickly determine the effect of pH changes and salt concentration changes in inorganic mercury solubility. For example, if inorganic mercury salts were placed in an alkaline waste basin at pH 10 or higher, HgO would precipitate, resulting in a total dissolved mercury level well under 1 mg/l at 200 mg/l $[Cl^-]$ (point A of Figure I-1) or 2000 mg/l $[Cl^-]$ (point C). If an alkaline waste basin at 20 mg/l $[Cl^-]$ (point A) were neutralized and brought to pH 7.6 (point B), the HgO(s) would redissolve, thereby raising the dissolved mercury level from 2×10^{-4} mg/l to approximately 20 mg/l. If an alkaline waste basin at 200 mg/l $[Cl^-]$ (point C) were neutralized and brought to pH 7.6 (point D) the HgO(s) would redissolve and could raise the dissolved mercury level from 2×10^{-2} mg/l to as high as 2000 mg/l. Thus, natural or man-induced changes in pH or chloride level can significantly affect the solubility (and thus the mobility) of inorganic mercury at waste disposal sites.

The equilibrium constant in the literature (6) for the reaction

$$Hg(OAc)_2 + 2\ H^+ = Hg^{+2} + 2\ HOAc$$

in 25°C water is $10^{-3.11}$. To determine the extent of hydrolysis, the acetic acid-water equilibrium has to be considered. Given the K_a of acetic acid of 1.8×10^{-5} at 25°C, the reaction

$$Hg(OAc)_2 = Hg^{+2} + 2\ OAc^-$$

has an equilibrium constant of 2.5×10^{-13}.

Mercuric acetate in benzene or acetic acid solution is capable of undergoing photolysis by ultraviolet light. An abstract review of a 1960 article by Ol'dekop, et al. (8) proposed the mechanism:

$$Hg(OAc)_2 + h\nu \rightarrow AcO\cdot + \cdot HgOAc$$

$$AcO\cdot \rightarrow CH_3\cdot + CO_2$$

$$CH_3\cdot + Hg(OAc)_2 \rightarrow CH_3HgOAc + AcO.$$

$$2\ \cdot HgOAc \rightarrow Hg_2(OAc)_2$$

$$Hg_2(OAc)_2 + h\nu \rightarrow Hg + Hg(OAc)_2$$

$$CH_3HgOAc + h\nu \rightarrow CH_3\cdot + \cdot HgOAc$$

$$2\ CH_3\cdot \rightarrow C_2H_6$$

ANALYTICAL METHODS

The literature contains an extensive number of analytical methods for mercury and for the content of mercury compounds in water, soil and biological materials. Several of the more sensitive methods are reviewed in articles by Baker and Luh in 1971 (9), by Ward in 1970(10), in a 1972 monograph by D'Itri (2), in a 1972 book edited by Friberg and Vostal (4), in a 1973 review by Reimers, Burrows and Krenkel (11), and in a 1974 article by Uthe and Armstrong (12). Flameless atomic absorption spectroscopy is based on mercury absorbance of 253.7 nm light (9). Mercury ions are reduced by stannous sulfate in a hydroxylamine sulfate-sodium chloride solution followed by acidification with sulfuric acid-nitric acid solution. The metallic mercury is swept from solution by 1 liter/minute airflow through a quartz absorption cell. This method differs slightly from that recommended by the Environmental Protection Agency (EPA) in 1975 for total mercury (13) and described in their 1974 publication (14). In that method, potassium permanganate and potassium persulfate are first added to oxidize organomercury compounds and remove sulfide. This method can be applied to biological samples. Flameless atomic absorption spectroscopy methods can determine 0.2 ppb of mercury (0.2 µg/liter of a solution). A variation of the flameless atomic absorption method uses a tantalum boat (15). Ward (10) describes the atomic absorption method used by the U.S. Geological Survey laboratories for mercury content of soils and rocks. The sample is heated to about 500°C in a radio frequency field to drive off mercury and particulate or vapor oxidation products. The mercury is trapped by amalgamation on a gold or silver leaf; other evolved products are shunted out of the system. The radio frequency field is changed to heat the gold or silver foil, and the vaporized mercury is directed to a measuring chamber where its ultraviolet absorption is sensed by a photocell. The decrease in light intensity is related to the amount of mercury vapor. The method is considered accurate to 1 ppb. An ingenious system for collecting the vapors of metallic mercury and organic mercurials from aqueous solution utilizes a rubber diffusion membrane and an argon stream. The vapors are detected through measurement of the intensity of the 253.7 nm mercury emission line excited by dc discharge (16). The sensitivity of 0.004 ppb makes this one of the most sensitive methods of analysis.

Dithizone ($C_6H_5N{=}N{-}(C{=}S){-}NHNHC_6H_5$) complex formation with mercury is widely used as a wet chemical technique. As described by Baker and Luh (9), the sample is first digested with a sulfuric acid-nitric acid solution or a sulfuric acid-potassium permanganate solution. Dithizone in chloroform is added, and a yellow-orange complex is formed, which extracts into the chloroform phase. According to Friberg and Vostal (4), measurement with 490 nm light is most sensitive. A limit of detection of 0.01 mg/liter is considered typical, although 0.001 mg/liter (1 ppb) has been claimed (9).

Neutron activation analysis (17) is extremely sensitive, but requires much time, a skilled analyst, and access to a thermal neutron reactor. The analysis presented below is for mercury only; several analyses exist where different metals are separated from each other after neutron exposure and each counted for a distinct isotope. In 1971, Pillay et al. (18) described a mercury analysis suitable for biological or environmental sources. He gave procedures for the preparation of biological tissue, plankton or algae, and sediment or silt samples. Samples are placed in polyethylene bags and exposed to 5 x 10^{12} neutron/cm^2-sec flux for two hours. After exposure, a precise amount of mercury carrier is added, and the sample is wet ashed (if biological) or digested (if soil or silt). Mercury is precipitated as the sulfide and then dissolved in aqua regia. The mercury is then collected by electrolysis on a gold cathode. Radiation from the cathode is then counted for ^{197}Hg emissions. The mercury content is determined by comparison between the sample and a known sample of mercury carried through the entire analysis. Accurate determinations were reported for 0.01 ppm mercury content, although the analysis is probably capable of detection to much lower concentrations.

Mercury vapor in air can be measured directly, or after sorption and desorption (19, 20), with ultraviolet spectroscopy; commercial instrumentation is available. Friberg and Vostal indicate that the sensitivity of such detectors is 2 $\mu g/m^3$ (4). If particulate matter is in the air, the sample can be collected in a bubbler with potassium permanganate-sulfuric acid solution and then processed either colorimetrically or by atomic absorption. An alternative is the use of selenium sulfide test paper, which stains brown when exposed to mercury vapor. Such a method was described by Sergeant et al. in 1957 (21). A combination activated carbon-mineral wool fiber trap is used to collect vapor and particulates. The carbon used is impregnated with iodoform to enhance mercury adsorption. After the collection step, the carbon and fiber are put into an ignition tube. If the presence of mercuric chloride, along with metallic mercury is suspected, additional carbon and iron filings are added to reduce the salt. Sodium oxalate is used to generate carbon monoxide, which forms when the ignition tube is heated and sweeps mercury vapor to the test paper. This method can detect 100 $\mu g/m^3$ of mercury in air.

A thin-layer chromatographic technique was reported by Lingle and Hermann in 1975 (22) to identify $HgCl_2$, CH_3HgCl and C_6H_5HgCl in partially digested municipal sludges. Mercurials were extracted from the sludge with cysteine hydrochloride-isopropyl alcohol solution, with mixing by an ultrasonic probe. The mixture was extracted with 0.004% dithizone in ether. After formation of the mercury complex in ether, concentration and drying, 15 microliters of ether solution was spotted on a thin-layer chromatographic plate. The plate was immersed in a 19:1 petroleum ether-acetone solvent to separate the already visible spots. The method was capable of detecting 3 μg of $HgCl_2$ in a 25-g sample of sludge.

Gas chromatography can be used for organomercurials; inorganic mercury can be converted to organomercurials to permit versatile use of the technique down to about 10 ppb (23). In a 1974 article by Jones and Nickless (24), a method was presented for the detection of inorganic mercury by methylation of such compounds with sodium 2,2-dimethyl-2-silapentane-5-sulfonate. The resulting methylmercury compounds can be analyzed by a gas chromatograph with a ^{63}Ni source electron-capture detector. The method was used to analyze 0.1 g samples of fish and sediment and was capable of detection in the 2.5 ppb to 10 ppm range.

Other methods, discussed by Baker and Luh (9), were spot testing, ion exchange, complex formation with sodium diethyl dithiocarbamate or dimethyl- or diethyldiselenocarbamic acid, polarography, mass spectroscopy, and X-ray fluorescence. Ward (10) discusses flame atomic absorption, neutron activation and colorimetric methods more applicable to rocks and soils. Uthe and Armstrong's article (12) specifically addressed methods used to determine methylmercuric compounds separately from other (usually called "inorganic" mercury) compounds.

MAMMALIAN TOXICOLOGY

Human Exposures

Established limits for exposure to mercury or mercury salts represent a compromise between desirability and practicality (see "EXISTING STANDARDS" for specific values).

These limits were arrived at by estimation from human experience and from analytical determinations of mercury levels in hair and blood of individuals who had consumed mercury in fish. The lowest mercury levels reported to have been present in persons showing neurological signs of toxicity were 0.05 mg/g in hair and 0.0002 mg/g in blood or 0.0004 mg/g in blood cells (25). Skerfving in 1973 (26) reported marginally increased chromosome aberrations in a group of 5 females and 18 males exposed to mercury by consuming three or more meals per week for over three years of fish containing 0.5 to 7 ppm of methylmercury.* Blood cell levels in all but one case were below the 0.0004 mg/g limit, with four cases between 0.0003 mg/g and 0.0004 mg/g and the remainder below 0.0003 mg/g. It has been estimated that to attain the 0.0004 mg/g blood cell level requires the daily intake of 0.3 mg of mercury over a prolonged period (25). To provide a measure of safety in view of the above reported chromosomal effects and the as-yet largely unquantitated embryopathic effects from mercury ingestion (26), the World Health Organization has recommended a provisional tolerable intake of

*Methylmercury represents those compounds where a methyl group is directly bonded to mercury, for example, methylmercuric chloride or dimethylmercury. There is no specific compound called methylmercury.

0.3 mg/week (25, 27). In perspective, the frequency of chromosome aberrations ascribed to mercury is lower than that seen following exposure to therapeutic X-rays, viruses, or lead (26); however, the medical significance of such damage is unknown.

Inasmuch as mercury can enter the body by oral, dermal, and inhalation routes, the above maximum tolerable weekly intake must include mercury derived from all three routes. While the amounts of absorption will vary depending upon the compound of mercury involved and on the route, such differences become relatively unimportant at the very low levels that can produce minimal toxic effects. Furthermore, at these levels, the safest assumption is to treat any form of mercury as though it would be methylmercury. There is a growing body of evidence that the developing embryo is uniquely sensitive to mercury, so that infants born to women exposed to alkylmercury are deformed and mentally retarded. D'Itri, in his 1972 review (2), reports that to date 23 abnormal children have been identified whose mothers were exposed to methylmercury-contaminated fish during gestation. Only one of the 23 mothers had exhibited any signs of mercury intoxication. The red blood cell mercury concentration in the fetus runs 20-30% higher than in the mother.

The single dose toxicity of mercury and its compounds depends to a large extent upon the amount of the dose that is absorbed into the body. Elemental mercury by mouth has produced severe poisoning, but few immediate fatalities, because most of the mercury is excreted in the feces. On the other hand, a number of deaths have been recorded from a one-day exposure to mercury vapor. Bidstrup, in a 1964 review (28), estimated one gram of mercuric chloride by mouth to be the approximate LD_{50} for man, with 0.5 gram being usually non-fatal and 1.5 gram being almost surely fatal. Availability of BAL (British Anti-Lewisite) as an antidote has materially reduced the death rate from such doses. The immediate effects are referable to protein coagulation, irritation, and corrosion of tissues with subsequent vomiting, salivation, abdominal pain and edema of exposed tissues. Death usually is a result of kidney damage or corrosion of the large intestine, and may be delayed several days to several weeks.

Since the vast majority of human poisoning cases with alkylmercury compounds followed several weeks' to several years' exposure, no reliable estimate of the human LD_{50} is available.

Repeated exposure to mercury or its inorganic salts by inhalation, ingestion, dermal contact or some combination of these routes through occupational, accidental, or medical use has resulted in many tragic human poisoning episodes. Unfortunately, a toxic or lethal dose often accumulates before signs of toxicity are sufficiently marked to serve as a basis for diagnosing the cause. Elemental mercury and alkyl-mercury, being non-ionic, can cross the placental and blood-brain barrier to produce severe and lasting damage. Ionic forms of mercury

do not easily pass these barriers, and some metabolic conversion in the body may be required before appreciable mercury from these sources enters the brain or the fetus (2, 4). Signs of chronic poisoning by mercury or mercuric ions are progressively: salivation, gingivitis, stomatitis, emotional instability, and tremor. Kidney damage has also been observed in some cases.

The damage to the central nervous system produced by alkylmercury is expressed as ataxia, dysarthria, and constriction of the visual field. Excepting for signs of severe irritation to the gastrointestinal tract mucosa in acute cases, there is little to differentiate between signs of acute and chronic poisoning.

The toxicity of mercury and its compounds appears to be dependent upon the metabolism of the various compounds within the body. In man the half-life of mercuric ion is 37 days for females and 48 days for males, as determined by small doses of $^{203}Hg^{++}$ (4). For methylmercury the corresponding half-life is 70-74 days (29). Thus, an equivalent amount of mercury in the form of methylmercury is expected to be more toxic and more accumulative than inorganic mercury. This is borne out by the fact that most mercury in living organisms is in the form of methylmercury. Ali-Shahristani, in 1974 (30), analyzed the hair of 48 patients who had ingested seeds treated with methylmercury. He confirmed a mean half-life figure of 72 days but noted five individuals showing a 110-120 day half-life. Thus, possibly 10% of the population is at greater risk than would be indicated by the use of a 70-day half-life in setting allowable limits.

At the same time, it has been shown that arylmercury compounds are more easily converted to elemental mercury and inorganic mercury than is methylmercury (31). Finally, mechanisms exist, both enzymatic and non-enzymatic, that can convert either elemental or inorganic mercury to methylmercury. Gage, in 1975 (32), reported several mechanisms by which organic mercury compounds are metabolized to elemental and inorganic mercury. Ascorbic acid with traces of copper, soluble plasma proteins, and dialyzed rat liver homogenates are each capable of effecting breakdown to elemental mercury and inorganic mercury. Since inorganic mercury is eliminated more rapidly from the body than methylmercury, compounds that are most easily metabolized to inorganic mercury are the least toxic.

The routes of mercury excretion include exhalation of elemental mercury vapor, feces, urine, sweat, milk, and hair. The proportions appearing in each excretory pathway vary, depending upon the compound consumed, the time after last exposure, and with inorganic mercury compounds, the dose. For these reasons, blood or urine samples subsequent to exposure are poor indices of the extent of exposure to inorganic mercury. With organic mercury, especially methylmercury,

half-life and excretory patterns are such that red blood cell levels can be used to monitor excessive intake. Since the most likely form of human exposure to mercury via fish, eggs, meat, milk, and vegetables is as methylmercury, such an available monitoring mechanism is fortunate. Levels in the hair may also be used as an exposure index since there is about a 300 to 1 ratio between hair and whole blood (4). Review of available data, however, indicates this to be a less reliable index (33).

In spite of the many papers reviewed by Friberg and Vostal in 1972 (4) on the urinary and fecal routes of excretion of various compounds of mercury, a lot of confusion still exists. Evidence is cited to indicate that methylmercury is excreted via the bile and then subsequently is resorbed, whereas inorganic mercury is also excreted via the bile but is not so readily resorbed from the lower gastro-intestinal tract. Such an interpretation of the data would go a long way in clearing up the confusion.

Experimental Animals

A systematic investigation of the toxic properties of mercury and compounds of mercury in laboratory animals has in reality never been conducted, although there is a vast literature on the subject. This is likely a reflection of the many documented human poisoning cases that can be used directly for estimating exposure limits for man. Such animal toxicity data as do exist were obtained incidental to experiments designed for metabolic studies, mechanism studies, or studies on specific compounds developed for pesticide use.

Oral LD_{50} values as given in the 1974 Toxic Substances List (34) range from about 10 to 100 mg/kg, depending upon the compound and species. In general, inorganic mercury and methylmercury are most toxic and arylmercury compounds the least toxic within that range. This compares with a human LD_{50} of mercuric chloride of 16 mg/kg. Thus, the variation among species is not large (28). Rates of metabolism and elimination of mercury compounds vary among different species. In the case of methylmercury, comparative half-life values by species are: man, 70-74 days; monkey, 50-60 days; rat, 20 days; mouse, 6-7 days (4). A corresponding proportionate decrease in sensitivity to the toxic effects of methylmercury may be expected in these species, with the short half-life species the least sensitive.

Methylmercury dicyandiamide has been given in single oral doses to 16-28 kg pigs (35). No toxic signs were seen in two pigs given 2.5 mg/kg during a 32-day observation period. One of two pigs at 5 mg/kg developed moderate neurological signs after three weeks; three of four at 10 mg/kg exhibited mild to severe signs of toxicity, and at 20 mg/kg two of four died and a third was euthanatized in extremis. Methylmercury in the form of CERESAN L* was fed to one calf each

*CERESAN L (methylmercury 2,3-dihydroxypropyl mercaptide - 2.84%, methylmercuric acetate - 0.62%).

weighing about 50 kg initially for up to 91 days at 0.05, 0.1, 0.2, and 0.4 mg/kg/day as mercury (36). Ataxia progressing to convulsions and a moribund condition developed on day 91 at 0.2 mg/kg/day and on day 33 at 0.4 mg/kg/day. In neither the pig or calf study was the observation period long enough to be certain that signs of toxicity would not develop at the lower doses used. Methylmercury at 0.25 mg/kg/day as mercury, fed to cats produced ataxia, intention tremor, and convulsions after 76-100 days (37).

Compounds of mercury on occasion give rise to various dermatological disorders, most often eczematous sensitization. Magnusson and Kligman, 1969, (38) have experimentally produced such sensitization in guinea pigs and man as a predictor test.

Ramel (4) has cited conflicting dominant lethal experiments reported for mice and rats. Methylmercury is certainly embryocidal, as shown by Spyker 1973 (39). Embryopathic effects due to inorganic mercury have also been reported by Gale in 1974 (40) with hamsters. The lowest oral dose producing a significant difference between control animals and those receiving mercuric acetate was 8 mg/kg and this effect was the production of "small" embryos. Doses of 25 mg/kg and above were embryocidal. Spyker, 1973, (39) demonstrated embryocidal activity in mice using methylmercury by intraperitoneal injection at 2 mg/kg. Gale (40) reported similar activity in the hamster at the same dose and by the same route.

The teratogenic effect of mercury compounds has been amply demonstrated in man as a result of the many mass poisoning episodes during the past 20 years (2, 4, 41) and in rodents (39, 40).

Perhaps the most serious aspect of environmental mercury contamination is the potential for adverse effects on postnatal development of offspring exposed to mercury *in vitro*. Retarded childhood development, including mental retardation, was first noted in victims of the previously mentioned human poisoning episodes. Spyker (39, 41) has observed a number of behavioral and neurological deficits in mice exposed *in utero* to methylmercury. These mice appeared normal at weaning, but gradually developed deficits three to four months later. Offspring of treated mothers exhibited more signs of miscellaneous infections. The immune response mechanism was checked and it was found that the thymus-mediated immune system was unaffected, but that the B-cell immune system was significantly deficient. These effects have now been noted down to a single 0.1 mg/kg dose administered once to the mother during mid-gestation. Further definition of the effects of methylmercury on postnatal development of animals exposed *in utero* is obviously needed.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Transport as a Function of Chemical Species Transformations. The chemical behavior of mercury in distilled water was reviewed by Hem in 1970 (6). Specifically, he studied the species in aqueous solution at 25°C in the presence of up to $10^{-3}M$ sulfate, $10^{-3}M$ chloride and mercury, over the pH range 0 - 14 and the redox potential range -.8 to 1.2 volts. At pH from 5-9 and redox potential from 0 to 0.5 volts, conditions found in river or lake waters, dissolved metallic mercury is the stable species. At lower redox potentials, such as found in lake bottoms, the stable species is the rather insoluble mercuric sulfide (HgS). This is so insoluble that it can form in a variety of ways from mercury or its salts and sulfur or sulfides. Under such conditions, the mercury content in water would fall to 0.002 ppb as opposed to 25 ppb for dissolved mercury in aerated natural water.

The chemical behavior of inorganic mercury compounds dispersed into an aqueous environment in contact with atmospheric oxygen has been explained in detail in the section of this report on "PHYSICAL AND CHEMICAL PROPERTIES." The solubility of mercury compounds is a strong function of chloride concentration and pH.

In a 1970 article by Jenne (43), an example was given of mercury concentration change in river water from 136 ppb to 0.04 ppb in a 50-60 km reach. In an abstract review of a 1965 article by Beisova and Fasenko (44), such self-cleansing was attributed to formation of insoluble compounds such as HgS, to formation of complexes and to adsorption on suspended particles. It is estimated (45) that suspended matter in areas of industrial pollution may contain from five to 25 times as much mercury as the water around it.

Of particular concern is the formation of methylmercury* from inorganic mercurial in lake sediments. In a 1975 article by Lingle and Hermann (22) and a 1970 article by Greeson (46), some work in this field is reviewed. According to Lingle and Hermann's article (22), methylmercuric chloride formation was not detected when partially digested sludges were dosed with mercuric chloride and phenylmercuric chloride.

A 1974 paper that clarifies the transformations involved was published by Holm and Cox (47). In one experiment, pond sediment was dosed with a growth medium to which were added different levels of mercuric chloride and calcium acetate; the mixtures were incubated anaerobically for 25 days. The authors found that acetate ion was required for methylmercury production. Moreover, more elemental

*Methylmercury represents those compounds where a methyl group is directly bonded to mercury, for example, methylmercuric chloride or dimethylmercury. There is no specific compound called methylmercury.

mercury was produced (as found in sediment) than methylmercury. In a second experiment, pond sediment was dosed with a growth medium, mercuric chloride and calcium acetate, and incubated for 28 days; the first 14 under anaerobic conditions, the last 14 under aerobic conditions. During both periods, methylmercury was accumulated in sediment at a rate of about 5 ng/g/day. From the 250 mg of mercuric chloride added, about 120 µg of methylmercury was produced and 3 mg of mercury (elemental) was recovered in the atmosphere above the culture.

In a third experiment, elemental mercury was added to sediment and a 33-day anaerobic incubation period ensued. A control sediment (no mercury added), incubated for 33 days, had a 0.12 µg/g total mercury content with no detectable methylmercury. After 4 days' incubation, sediment dosed with mercury had a 1.12 µg/g total mercury content, of which 0.006 µg/g was methylmercury. After 33 days, the contents were 15.6 and 0.017 µg/g respectively.

Jenne (43) discussed atmospheric transport of mercury. When air at 17°C was saturated with mercury, a concentration of 10^6 ng/m^3 could be attained. However, air in areas without mercury deposits had 1-5 ng/m^3 mercury concentration, while soil air at mercury deposits could contain 100 ng/m^3 mercury or higher. Thus, the rate of exchange of soil air with atmospheric air is faster than the rate of evaporation of mercury to the soil surface.

Accumulation: The adsorption of mercury and mercurials into soils has been reviewed in 1970 by Jenne (43) and by Lagerwerff in 1972 (48). Jenne indicates that quantitative data on the subject are not well reported. In the second article (43), an unpublished study was cited, wherein 50-ml portions of 10-ppb solutions of mercuric ion were applied to 0.5g samples of each of three soils and of peat moss. From half to nearly all the mercuric ion was sorbed from all solutions in one hour; at least three quarters was sorbed from all solutions after 24 hours. Then the soils and peat moss were washed with either filtered tap water or 0.5N sodium chloride. The soils and peat moss lost from less than 1% to 5% of sorbed mercuric ion; slightly less was desorbed with salt solution.

Lagerwerff (48) indicated that "the retention of Hg in soil is due not only to valence-type ionic adsorption by organic and inorganic materials and the formation of covalent bands with organic compounds, but also to the low solubilities of Hg as phosphate, carbonate, and especially sulfide." He also indicated that aerially deposited mercury (intentional spraying, rainfall or fallout) is retained in the surface layers of soils.

This phenomenon was also reported by Anderson in 1967 (49). Analysis of a large number of Swedish and African topsoils indicated average mercury contents of 60.1 and 23.3 ng/g respectively. The subsoil portions of such soils ranged from 2 to 10 ng/g. Anderson studied the adsorption of mercury in water on either the humus-rich or mineral-rich fractions of soils. At pH 4-6, the humus-rich portion adsorbed the largest amount of mercury; at pH 7-9, the mineral-rich portion adsorbed the largest amount. The paper presented distribution coefficients of mercury between the soil components and water over the range of pH studied. For example, at pH 4.3, when humus contains 2.5 mg/g of mercury, water in equilibrium contains 3.1 µg/g mercury, 800 times less. As the concentration of mercury in soil decreases, the distribution coefficients increase.

In light of Anderson's work (49), the ability of soil to retain mercury can be illustrated by a study of 36 drainage water samples collected from Swedish fields, and reported by Lagerwerff (48). The mercury content of these samples ranged from 0.02 to 0.07 ppb. In 1962, Ross and Stewart (50) reported on soil (unspecified) core assays performed in 1960 after 21 spray applications of phenylmercuric chloride from 1954 to 1958. The mercury was concentrated in the top two inches of soil, approximately 6 times as much as was found in the 2-to 4-inch core. Only "trace" amounts were found in the 4-to 6-inch core.

In 1967, Aomine and Inoue (51) reported on mercury retention in three Japanese soils, each with a different type clay. They determined equilibrium data for the adsorption of mercuric chloride and phenylmercuric acetate, and desorption from the soils of phenylmercuric acetate. Solutions with mercury concentrations of 100 to 1000 ppm were used. Generally, higher adsorption of mercury occurred with phenylmercuric acetate solution than with mercuric chloride solution. Generally, montmorillonite clay had the highest adsorptive capacity; contradictory results were reported for overall soil adsorption and clay-fraction adsorption.

In 1969, Inoue and Aomine (52) reported on further studies using lower concentrations of phenylmercuric acetate. They found pH dependency of the clay portions of soil with maximum adsorption around pH 6, whereas in Anderson's study (49), maximum adsorption occurred at pH 8. Moreover, the organic portions of the soils contributed more to the overall adsorption of soils than in their 1967 (51) study.

Background Concentrations: The source of mercury in soils appears, in many cases, to be geological. All rocks contain mercury; generally the range is from 50 to 500 ppb (4) but can attain anomalously higher levels. Estimates of background levels in soil vary somewhat:

10-1000 ppb (53); 10-500 ppb (10, 48); average of 71 or 100 ppb in the U.S.A. (48); average of 55 ppb for Western U.S.A. (48); 96 ppb for Eastern U.S.A. (48); 30 to 500 ppb for typical soils (45); and 10 to 150 ppb for typical soils (2). Exceptionally high mercury levels, apparently of geological origin, were found in several British garden soils, namely 250 to 15,000 ppb (54). High mercury concentrations are also found in certain other parts of the world (4). Pierce, Botbol and Learned (55, 56) considered that rocks and stream sediments containing more than 1000 ppb, and soil containing more than 500 ppb of mercury in soils of Western United States would be "worthy of further investigation as possible results of (1) mercury mineralization processes or (2) surface contamination by mercury-bearing wastes."

The concentration of mercury in surface waters is generally low. The normal range has been given as 0.01 - 0.05 ppb for rivers and groundwater (53) and as 0.02 to 0.7 ppb, but generally less than 0.1 ppb (2). A survey of 73 samples (57) from 31 states showed a range from less than 0.1 ppb to 17 ppb. Of these samples only 12 had a mercury content above 1 ppb, while 34 contained less than 0.1 ppb (the limit of detection). The mercury content of stream waters tends to decrease as the water moves away from the mercury source, owing to sorption, dilution, vaporization and precipitation (43, 45).

Mercury enters the atmosphere as particulates and as metallic mercury vapor, dimethyl mercury, and, to a lesser extent, certain somewhat volatile undissociated salts (43). The principal modes of return to the earth are through solution in rainwater (43) or through adsorption on settling particulates (43). An estimate for mercury deposition in Sweden by rainfall was 0.48 gram/acre/year (43). If this were accomplished at one time, it would approximate the level normally used for fungicidal treatment of the soil. As mentioned above (43), air in areas without mercury deposits contains 1-5 ng/m^3 of mercury.

Animals

Mammals: The data on mammals are covered under the section on *"Experimental Animals."*

Birds: Experimental feeding of mercury compounds to kestrels (Falco tinnuculus) revealed, on autopsy, 49 to 122 ppm of mercury in liver and 20 to 33 ppm mercury in brain tissue (58). One year-old red-tailed hawks (Buteo jamaicensis) that were fed chicks containing 3.9 to 10 ppm methylmercuric ion (abbreviated $MeHg^+$) in their livers for 4 to 12 weeks died after showing neurological symptoms (59). Goshawks (Accipiter g. gentilis) fed tissues of chickens which averaged 13 ppm of mercury had gonadal methylmercury concentrations of 280 ppm (60). Seed treatment with mercury and use of the seed as food resulted in accumulation of mercury in the

organs of pheasants (61). Treated seed fed to chickens caused mercury to pass into their eggs (48). High dietary intake of mercury (as mercuric nitrate) resulted in poor reproductive efficiency in wood pigeons, decreased hatchability of pheasant eggs, and the appearance of methylmercury in tissues and eggs of hens (62). Retention time of mercury in tissues of ducks was greater than in pheasants and chickens; the least in chickens (63).

Amphibians: A salamander was found with 0.5 ppm of mercury in the heart muscle and 9.2 ppm in the liver when the typical soil level was 10-20 ppm. Other data given in the same reference were not correlated with soil levels of mercury, but generally showed high mercury levels in amphibians from mercuriferous areas (64).

Fish: Mercury produced measurable behavioral changes in goldfish at 0.003 ppm (65). Although it has been reported that mercury is "infinitely" toxic to fish (66), bioconcentration does occur in fish and can result in human poisonings due to ingestion of contaminated fish (2). The bioconcentration factor in fish has been estimated at 10^3 to 3×10^3 (48).

Apparently the reason that fish do not always die from the mercury is that the toxin is mainly (50% of $MeHg^+$ ingested) deposited in muscle tissue, with much less accumulation in the vital organs (e.g. 0.1% of ingested $MeHg^+$ is found in the brain). Thus, fish not yet incapacitated can have dangerously high concentrations of $MeHg^+$ in their muscle tissue. Daily consumption of fish with 5 to 6 ppm of mercury may be lethal to humans (67). Mercury can also be taken up by fish directly from the water (68).

The ability of $MeHg^+$ to cross biological membranes, its small size, single valence, and high solubility in lipids results in accumulation in brain and nerve tissue (67).

Accumulation of $MeHg^+$ in fish depends on the amount of intake, the retention time (67) and the age of the fish (69). Intake depends on availability in the environment, while retention time varies between species. Half-retention time (time required to excrete one-half of the ingested mercury) in rainbow trout (Salmo gairdneri) is over 200 days; for pike, over 600 days (67). As the fish ages, there is a linear increase in the percentage of $MeHg^+$ deposition in relation to total mercury intake (69).

Invertebrates: Freshwater invertebrates may concentrate mercury by a factor of 10^5 (48). Mercuric oxide is the most toxic form to flatworms (Planaria) (70). Drosophila fed 0.25 ppm mercury had a variety of genetic defects (71).

Microorganisms: Inorganic mercury compounds have long been utilized as fungicides and bactericides. Susceptibility to mercury is quite variable among microorganisms. Ashworth and Amin, 1964 (72) observed that Aspergillus niger was resistant to methylmercury poisoning because of nonprotein intracellular sulfhydryl compounds that complex mercury and protected cell enzyme systems. Rhizoctonia solani and Pythium ultimum were not protected from mercury.

Tiwari, 1974, (73) studied the effects of various concentrations of mercuric chloride on soil microflora. Mercuric chloride concentrations in the range tested (0.1 to 0.5 gm per 100 gm soil) were toxic for Penicillium citrinum and Aspergillus candidus for a 30-day exposure period. Tiwari observed that both fungi developed resistance, as expressed by increased cell populations, when incubated with mercuric chloride for a 60-day period. Domsch (74) studied the use of various antibiotic substances on soil to control soil fungi. He concluded that mercuric chloride and other fungicides were only effective for an initial application period. Tolerant organisms soon became prevalent, thus demonstrating that mercurial antibiotics had only limited use in soil systems.

Jensen and Jernelov, in 1969 (75), were the first to demonstrate that, in aquatic sediments, $HgCl_2$ can be biologically methylated to CH_3Hg^+. Since that time other microbially initiated conversions of mercury have been discovered and the aquatic elemental mercury cycle has been elucidated (77). When inorganic mercury (Hg^o) is incubated with lake sediments, for example, both monomethyl (CH_3Hg^+) and dimethyl mercury [$Hg(CH_3)_2$] are formed as a result of microbial activity (77, 78, 48). However, microbial demethylation of mercury has also been observed, which results in the production of volatile elemental Hg^o in aquatic systems (78, 47). In anaerobic water-sediment systems to which $HgCl_2$ (Hg^{+2}) has been added, both Hg^o and $HgCH_3^+$ are produced biologically. Aerobic bacteria and fungi have been shown to methylate mercury as well (48). Certain aerobic bacteria in the genus Pseudomonas have been shown to convert various mercurials to volatile Hg^o and have, in fact, been used to volatilize, and thereby remove, mercurials from industrial wastes (48).

The complete aquatic elemental Hg cycle has been described very well by John Wood (77). Anaerobically, certain microbes can methylate or dimethylate Hg^o to form $HgCH_3^+$ or $Hg(CH_3)_2$. Other bacteria can convert ionic Hg^{+2} to either $HgCH_3^+$, $Hg(CH_3)_2$, or Hg^o. Both Hg^o and $Hg(CH_3)_2$ are volatile and can escape to the aerobic water column or to the atmosphere where further conversions can take place. By photolysis in the presence of ultraviolet light, for example, $Hg(CH_3)_2$ can be decomposed to Hg^o. Elemental Hg^o can then re-enter the sediments for further conversions. All of these interconversions seem to be in a dyanamic equilibrium of reversible reactions which leads to a more or less steady state concentration of $HgCH_3^+$ in aquatic sediments (77).

Izaki et al., 1974, (80) studied mercuric chloride resistance in Escherichia coli. They purified a mercuric ion-reducing enzyme which appeared to contain a flavin compound. FAD (flavin adenine dinucleotide) was found to be a component of the enzyme responsible for oxidation of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) and was dependent on mercuric ions. Rapid oxidation was noted in the presence of purified enzyme and mercuric chloride. Nonprotein SH compounds were essential for enzyme activity. Escherichia coli, Pseudomonas fluorescens, P. aeruginosa, Citrobacter, Bacillus megaterium and B. subtilis have been shown to oxidize elemental mercury which accumulates on the bacterial cells as a Hg^{+2} complex (47).

Nelson et al., in 1973 (81), utilizing mercury-sensitive Pseudomonas, demonstrated changes in morphology when cells were incubated with mercuric chloride. When the cells were grown in the presence of 15 ppm mercuric chloride, very small protoplasts were formed within a common cell wall. A mercury-resistant strain showed no obvious morphological changes when grown in 25 ppm of mercuric chloride. A sensitive Bacillus sp. incubated in 5 ppm mercuric chloride was observed by electron microscopy to have a condensed nuclear region and altered septum formation. Enterobacter also showed abnormal morphology in the presence of mercuric chloride (82). The plastids in the bacteria, including Staphylococcus, could mediate resistance to mercury. Bacteria seem to be capable of intra- as well as inter-generic transfer of the resistance factor (82).

Mammalian tissue culture cells (L-929) exhibit similar morphological alterations as observed in sensitive bacteria in the presence of as little as 0.5 ppm of mercuric chloride (83). The cells became large and multinucleate. At the same time, they were impaired in cell division processes, and active cell structures such as ribosomes and endoplasmic reticulum were reduced in number. Acid phosphatase activity was eliminated.

For species which have been examined, maximal tolerable doses of mercuric chloride, permitting growth, vary between species from 5 ppm for Pseudomonas fluorescens to 20 ppm for Myobacterium, Bacillus megatarium, Escherichia coli, and to 40 ppm for Aspergillus niger and Scapulariopsis brevicaulis (84).

Plants

Phytotoxicity: Mercury is known to injure many species of plants. Whether the mercury is in the form of elemental mercury vapor or of mercury compounds in the soil, it can inhibit plant growth and development (85, 86, 87).

There are considerable differences in species susceptibility to mercury. Roses are known to be very sensitive to mercury vapor (88). Beans, butterfly weed, Oxalis, and sunflower have also been demonstrated to be

very susceptible to mercury vapor while Aloe, Croton, and Sarcococca are not susceptible to mercury vapor (85). In general, the amount of plant injury from mercury vapor is related to concentration of the mercury and the length of time the plants are subjected to the vapor (85). Organic matter in soil can speed reduction of organic and inorganic mercury compounds to metallic mercury, leading to more mercury vapor and thus possibly to more plant injury (85).

Mercury compounds in soil seem more phytotoxic to dicotyledons than to monocotyledons. Booer (87), indicates that growth rates of oats, barley, wheat and lawn grasses are only slightly retarded by mercury in soil while lettuce and carrots are very sensitive.

Fresh weight increases in lettuce, carrots, cauliflower, and potato explants have been shown to be affected by concentrations of mercury (as $HgCl_2$) of 0.5 to 5.0 mg/l (86). Growth of mosses and lichens is retarded by mercury and mercury compounds in soil (87). Concentrations of $10^{-4}M$ cause a breakdown of cell permeability and alter cation transport in Chlorella pyrenoidosa (89). Organic mercury compounds, including methylmercury, reduce photosynthesis in phytoplankton at concentrations of less than 0.1 ppb (90).

Bioaccumulation: Mercury can be accumulated by plants from soil by root uptake of Hg^0, monomethyl Hg, Hg ions, or organic Hg, absorption into foliage of Hg vapor given off by soil or soil particles on aerial plant parts or gaseous exchange of Hg^0 through stomata (48, 72, 91, 92).

The amount of mercury accumulated by plants depends upon the mercury source, concentration, and species of plant. Shacklette (93) reported red cedar trees accumulate less than 500 ppb from soil containing up to 650 ppb. Alder, black spruce, birch, Labrador tea, and Spiraea accumulated 500 to 3,500 ppb mercury in leaves and stems (dry weight basis) if their roots were in direct contact with a cinnabar vein.

In a study on sludge application, Van Loon (94) found concentrations of 6 ppm Hg in bean seed pods and 12.2 ppm (dry weight basis) in red tomato fruit growing on a sludge-soil mixture containing about 15 ppm Hg. Control samples from plants growing on soil had 0.24 ppm Hg accumulation. Gracev and Steward (95) tested the mercury content of crops growing on soil containing less than 40 ng Hg/g soil and found that in some cases alfalfa, barley, wheat, oats, and rutabaga accumulated more than 40 ng Hg/g dry weight of plant tissues. The grain of cereals contained less Hg than the straw (95). Ross and Stewart (50) found no mercury residues in apple trees growing in soil to which phenylmercuric acetate had been added (mercury content of soil, up to 1800 ppb). Absorption of mercury by pea roots from solution of mercuric acetate and phenylmercuric acetate increased with increased mercury concentration (solutions of 1, 4 and 10 x $10^{-5}M$ Hg acetate had residues to 0.03, 0.21 and 0.47 µmoles Hg^{2+} respectively, per 10 roots) (91). Absorption increased with

temperature up to 37°C and mercury became distributed throughout the cellular fractions (91). In a study on mercury accumulation in vegetable and oat plants, highest accumulations in edible fractions were found in radish tubers (0.663 ppm accumulation from soil containing 20 µg mercury per gram of soil) (96). There were significant increases in mercury content of roots in carrots, peaks, cauliflower and spinach; in leaves of spinach, and carrots; in pods of peas; and in stalks of oats (96).

Contamination of foliage with mercury compounds in dust or rain can lead to accumulation of mercury by plants. Leaves, twigs, and shoots of trees accumulate mercury in urban areas, undoubtedly from airborne industrial wastes containing mercury compounds (97). Bryophytes in urban areas accumulate up to 2000 ppb mercury (98).

Smart (99) presented evidence of mercury compound translocation in many species of plants and indicated that organic mercury compounds are rapidly translocated. A review by Lagerwerff (48) indicates little uptake of mercury from soil treated with $HgCl_2$ or $HgCl_2$ in broccoli, carrots, potatoes, lettuce and beans. Autoradiographic studies have clearly indicated that radioactive mercury supplies to Mentha spicata in form of mercuric chloride or acetate solution around the roots could be translocated to the leaves (89). Apple trees accumulate mercury in fruit by translocation from leaves (50, 100).

Characteristics of the soil influence mercury accumulation. The solubility and availability of mercury in the soil for uptake by plant roots have been evaluated by Hahne and Kroontje (101). The mercuric ion hydrolyzes at low pH values and forms chloride complexes. Hydrous iron and aluminum oxide in soil favor immobilization of Hg (II) at pH levels below 5 (101). Jones and Hinesley (102) in summarizing the levels of mercury in crops growing on the Morrow soil plots over 63 years, reported a decline with addition of tile drainage. The retention of mercury in soils is due to interaction as insoluble salts and valence-type ionic absorption on organic and inorganic material plus formation of covalent bonds with organic material (48). Evaluation of beans, cabbage, carrots, millet, onions, potatoes, and tomatoes growing on different soil types containing mercury compounds (including mercuric chloride) indicates that accumulation was greatest in plants growing on a Howard gravelly loam, whereas there was very little accumulation from Oswego muck.

Mercury accumulation could come from mercury vapor absorption through leaves or uptake by roots from soil. In studies with turfgrass, Gilmour and Miller (92) found that 56 percent of mercury added as chloride salt volatilized during a growing season. Treatment of turfgrass with mercurous-mercuric chloride (1.2 g per square meter) at either the surface or in the root growth zone leads to leaf accumulation of 300 or 1.5 ppm Hg, respectively. Mercury translocated to foliage

decreased with time as applied mercury became unavailable. Lee (103) tested mercury uptake by wheat and barley plants from soil treated with 0.5 µg Hg/g soil. In the heading-out stage, wheat (above ground portion) accumulated up to 0.027 percent of applied mercury and barley (above-ground portion) accumulated up to 0.023 percent of applied mercury. Mature plants accumulated less than young plants.

Differences in mercury accumulation by edible fractions of different plant species are readily apparent in comparing plants growing in the same soil type containing the same amounts of mercury (Table I-4).

Mercury accumulation has been found in lettuce, radish, carrot and parsley after application of Cerezán* to soil (105). Bioaccumulation by algae (especially filamentous) has also been shown (106).

TABLE I-4. Accumulation of Total Mercury in Edible Fractions of Plants from Application of 10 ppm Mercuric Chloride to Howard Gravelly Loam Soil (104).

Plants	Accumulation (ppm)
Bean	6
Cabbage	43
Carrots	73
Onions	1087
Potatoes	130
Tomatoes	13
Millet	64

*Cerezán is a solution containing 10% methoxyethylmercury chloride.

Food Chain

The bioconcentration of mercury in aquatic species constitutes the greatest opportunity for mercury exposure. A large predatory fish that feeds on smaller fish could conceivably concentrate ambient mercury by a factor of 10^{11} (invertebrate 10^5 → small fish 10^3 → large fish 10^3, total 10^{11}). Mercury could be concentrated similarly in terrestrial food chains in animals raised on fish-based diets. "... legal-size hatchery fish treated with mercurials (for diseases) or wild fish that have eaten mercury-contaminated fingerlings may be a public health hazard" (107). Methylmercury levels in aquatic vertebrate and invertebrate predatory species were higher than in other less predatory or non-predatory biota (47).

Terrestrial birds have been shown to build up high mercury levels when eating seed treated with mercury compounds. Bird-eating falcons had high mercury levels in their eggs. Eagles and hawks that fed primarily on rodents did not have as high mercury concentrations. Thus, food habits seemed to predispose the level of acquisition of dietary mercury (108).

EXISTING STANDARDS

Mercury standards for industrial exposure (as TLV's), inhalation, water consumption and food ingestion are presented in Table I-5.

TABLE I-5. Mercury Exposure Limits for Humans.

		Ref.
LV - Inorganic vapor and salts; organic compounds other than alkyl mercury	0.05 mg/m^3	(109)
NIOSH - Inorganic mercury	0.05 mg/m^3	(110)
Alkyl mercury	0.01 mg/m^3	(109)
USSR recommended limit, inorganic	0.01 mg/m^3	(109)
Air Quality Standards - EPA	0.001 mg/m^3	(111)
Water Quality Standards - EPA	0.002 mg/L	(13)
- WHO	0.001 mg/L	(25)
Fish - FDA	0.5 mg/kg	(112)
Provisional Tolerable Weekly Intake FAO/WHO Expert Committee on Food Additives	0.300 mg/ person	(25, 27)

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APPENDIX J

DICYCLOPENTADIENE

ALTERNATIVE NAMES

Dicyclopentadiene; Bicyclopentadiene; Biscyclopentadiene;
3a,4,7,7a-Tetrahydro-4,7-methanoindene

PHYSICAL AND CHEMICAL PROPERTIES

CAS Reg. No. 0077-73-6
Toxic Substances List: PC 10500
Molecular formula: $C_{10}H_{12}$

Dicyclopentadiene (DCPD) is a waxy solid at room temperature with a strong camphor-like odor. The structures of DCPD appear in Figure J-1. The isomers may be considered as cis- and trans- in terms of the 2- and 8-carbons. Trans-DCPD is the usual form, (and where DCPD is cited the trans-form is understood). The cis- form has been prepared from the trans-form by a method described by Schröder (1). A 20% solution of DCPD in CS_2 was heated for 2 to 4 hours at 180°-200°C under 50 atmospheres pressure. The solvent was then distilled, and the products separated by in vacuo distillation with a 30% yield of cis-DCPD.

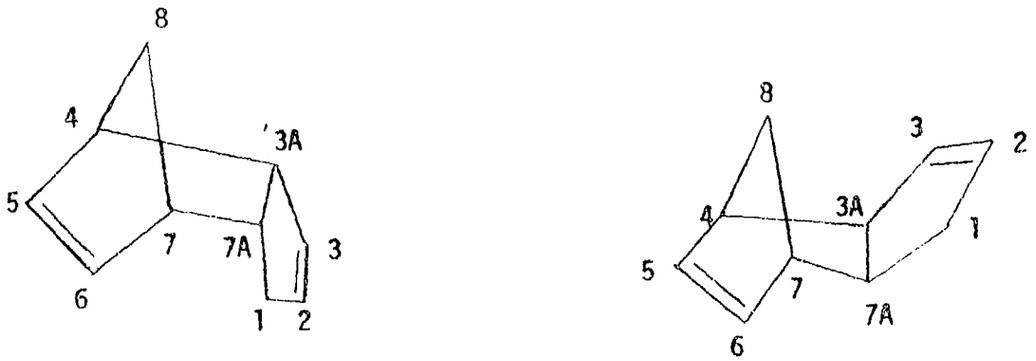


Figure J-1. Cis- and Trans- Isomers of Dicyclopentadiene

According to an article by Waring and co-workers (2), crude DCPD was distilled, and 40°-44°C range distillate (probably monomer) collected and allowed to stand overnight. The cis- isomer reportedly crystallized when the distillate was placed in vacuum, had a melting point of 27.8°C, and only a faint odor.

Table J-1 summarizes physical properties of trans-DCPD.

TABLE J-1

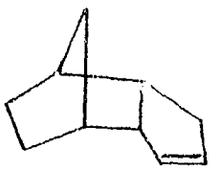
Physical properties of Trans-Dicyclopentadiene

Property	Value	References
Density at 20°C, g/cc	0.982	(3)
Melting Point, °C	32.9	(4)
n_D^{35} (Refractive Index)	1.5050	(5)
Temp, °C for cited Vapor Press, mm Hg		
20°	1.4	(3)
47.6°	10	(6)
105°	100	(3)
166.6° (boiling point)	760	(4)
Solubility in Water (ppm)	Considered insoluble Estimated 40*	(6) (6a)

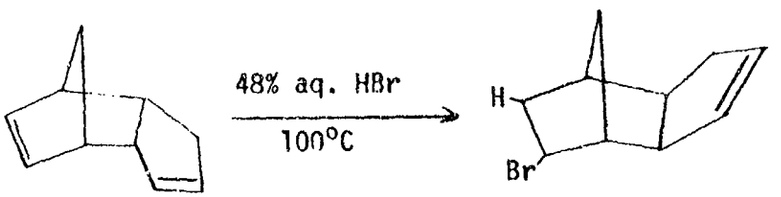
*Estimation on basis of solubility of diolefins of similar molecular volume (6a).

Dicyclopentadiene can be prepared by dimerizing cyclopentadiene. Harkness *et al.* (7) reported that the second-order rate constant for the reaction in liquid cyclopentadiene was $8.5 \times 10^7 e^{-14900/RT}$ cc/mole-sec. Thus, if one cc of cyclopentadiene is allowed to stand at 25°C for one day, 52% conversion to the dimer should occur. This result is approximate since the liquid phase changes density as dimerization proceeds. DCPD in turn can break down to the monomer. These authors (7) determined the breakdown to be a first-order reaction in the vapor phase with rate constant $10^{13} e^{-33,700/RT}$ sec^{-1}. In 1936, Khambata and Wassermann (8) reported the liquid phase rate constant as $3 \times 10^{13} e^{-35400/RT}$ sec^{-1}. At 30°C, the half-lives of DCPD breakdown in the vapor and liquid phases are calculated at 4,315 and 24,200 years, respectively. This means that the equilbrium between the monomer and dimer of cyclopentadiene lies strongly on the side of the dimer at ambient temperatures. The 584Å photoelectron spectrum of DCPD was measured by Baker *et al.* (9). DCPD undergoes reactions involving its double bonds; some of these were studied in a thesis by Donaldson in 1958 (10). If addition is made across one

double bond, it is inevitably the 5,6-bond. DCPD can be hydrogenated in the presence of Raney nickel to



However, typical addition reactions such as hydrohalogenation, hydration or esterification yield rearranged structures which are cis-oriented. For example:



Donaldson (10) found that 99% of the saturated analogue of DCPD, trans-4, 7-methanoindan, could be isomerized to the cis-4,7-methanoindan, in sharp contrast to DCPD. He presented the infrared spectrum of DCPD (identified as Spectrum #46) and spectra of the other compounds studied. Although he did not investigate the reaction of HOCl with DCPD, he indicated that addition occurs across the 5,6-bond to form a chlorohydrin (10).

ANALYTICAL METHODS

Until recently, analysis for DCPD as a trace pollutant was not well developed. According to a 1967 article by Szewczyk (11), DCPD absorbs in the infrared at 677 and 1344 cm^{-1} sufficiently distinct from the cyclopentadiene peaks of 644 and 1369 cm^{-1} to permit analysis of DCPD in the presence of cyclopentadiene. Raman spectrum frequencies are found at 1571 and 1613 cm^{-1} (12). Miskalis (13) used gas chromatography to detect DCPD in coke-oven gas. Gas chromatography of mixtures containing DCPD was reported as early as 1958 (14). Kinkead, *et al.* (3) used flame ionization gas chromatographic analysis (at 135°C, with a 10 ft column of 15% Tergitol NP-44 on Gas Chrom Q) to measure DCPD concentrations as low as 1 ppm in air. In work done for the Colorado State Department of Health, headspace analysis by gas chromatography was performed on water containing DCPD (15). Details of the analysis are not available, though it is claimed to detect DCPD at 0.28 ppb in water (16). DCPD in a benzene-acetic acid mixture gives a color text with bromine at a sensitivity of about 60 ppm (17). DCPD exhibits a fluorescence that might be useful for analysis (18).

MAMMALIAN TOXICOLOGY

There is no published information on the toxicity of DCPD to humans. Information on the mammalian toxicity of DCPD is summarized in Tables J-2 and J-3.

TABLE J-2

Summary of Acute Toxicity of DCPD

Animal Species	Route of Administration	LD50 (mg/kg)	Range Value	References
Rat	Oral	353	262-478	3
		410	310-530	19
Rat (male)	"	435	361-523	20
Rat (female)	"	396	343-458	20
Rat	Intraperitoneal	200		21
		310		3
Mouse	"	200		21
Rabbit	Dermal	5080*	3110-8290	3
		4460*	2440-8150	19
		6720*	3150-14360	22

* LC50 values.

TABLE J-3

Inhalation Toxicity of DCPD

Animal Species	Dose (ppm) & Exposure (hr)	LC50 (ppm)	Remarks	References
Rat	4	660	Range 553-817	3
"		359		3
"		385		3
"	Saturated vapor	1*		3
Mouse (male)	4	145		3
Rabbit (male)	4	771		3
Rat	2500/1		1/4 killed	23
"	2000/4		4/6 "	23
"	1000/4		4/4 "	23
"	500/4		1/6 "	23
"	250/6 x 10		1/4 "	23
"	100/6 x 15		4/4 "	23

* LT 50 (hr.)

DCPD was found to be an irritant when subjected to the standard rabbit eye irritation test but was not found to be a primary skin irritant (20). No TLV has been established for DCPD, but Gerarde (6) suggested "a value of 100 ppm seems reasonable based on the limited toxicity data available and extrapolation from similar chemicals." Kinkead *et al.* (3) have suggested a hygienic standard for man of 5 ppm. The TLV for DCPD recommended by Russian workers (24, 25), is 0.185 ppm (1 mg/m^3). Russian workers (26) have also recommended a permissible concentration of 0.0001 mg/l for DCPD in water supply systems. Man can detect 0.003 ppm DCPD vapor by odor (3).

The carcinogenicity of DCPD by intramuscular injection in the rat was investigated under an NCI contract at the Institute of Chemical Biology, San Francisco University (Dr. A. Furst, 1975) (27). The compound was not considered to be carcinogenic under the conditions of the experiment.

The toxicology of DCPD, including phytotoxicity, has been summarized in a fact sheet (28). The pathological effects in rats were typical of irritating hydrocarbons when administered orally in large doses; it is slightly to moderately toxic by the dermal route; and highly toxic by the oral and intraperitoneal routes in single dose studies. The lack of complete data indicates the need for further studies for an accurate evaluation of the toxic potential of DCPD. Recommendations for further toxicological studies have been made (28), and the implementation of these recommendations has already been undertaken through a USAMRDC contract with Litton Bionetics Inc., Falls Church, Virginia 22046.

ENVIRONMENTAL CONSIDERATIONS

No information is available as to DCPD behavior in soil and water, its effects on animals in the environment, or its transmission through food chains. A USAMRDC contract study to determine the toxicity of DCPD to aquatic vertebrates and invertebrates has been initiated through Bionomics, E. G. & G., Inc., Wareham, MA 02571.

Plants

Tests conducted at Ft. Detrick during 1974-1975, in which wheat (Wichita) and beans (Black Valentine) were treated with diisopropyl methylphosphonate (DIMP) and DCPD combined (water solution to soil), showed a greater effect on test plants than treatments with DIMP alone. Thus an additive, or possible synergistic, effect due to DCPD was suggested. Tests conducted with DCPD alone at 10 and 40 ppm caused tip burning of leaves (29). A USAMRDC contract study to determine plant uptake and effects and soil retention of DCPD has been initiated through Aerojet Ordnance and Manufacturing Co., Downey, CA 90241.

EXISTING STANDARDS

No information available.

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APPENDIX K

ALDRIN/DIELDRIN

Because aldrin is converted readily to dieldrin in the biosphere, and the two therefore often occur together, it is convenient to treat them as a subgroup, while showing their differences and similarities.

ALTERNATIVE NAMES

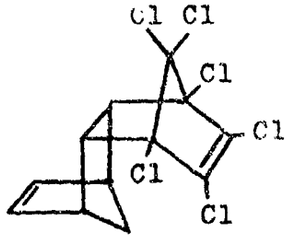
ALDRIN: 1,4:5,8-Dimethanonapthalene, 1,2,3,4,10-hexachloro-1,4,4a, 5,8,8a-hexahydro-, (1α,4α,4aβ,5α,8α,8aβ)- (Chem. Abstr. after 1961); aldrin (Chem. Abstr. before 1961); Aldrex; ENT 15,949; Compound 118; hexachloro-hexahydro-endo-exo-dimethanonapthalene; 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4-endo-5,8-dimethanonapthalene; HHDN; Octalene; Seedrin.

DIELDRIN: 2,7:3,6-Dimethanonapth[2,3-b]oxirene, 3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-, (1aα, 2β, 2aα, 3β, 6β, 6aα, 7β, 7aα)- (Chem. Abstr. after 1971); 1.4:5,8-dimethanonapthalene-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro, -endo, exo(Chem. Abstr. after 1961); dieldrin; Compound 497; ENT 16,225; Hexachloroepoxy-octahydro-endo,exo-dimethanonapthalene; HEOD; Illoxol; Octalox-Panoram D-31.

PHYSICAL AND CHEMICAL PROPERTIES

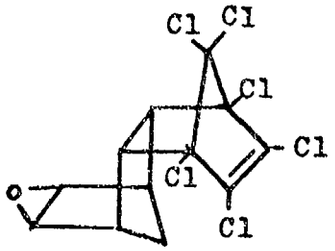
ALDRIN
CAS Reg. No. 309-00-2
Toxic Substances List: IO21000
Wiswesser Line Notation: L D5 C555 A D- EU JUTJ AG AG BG IG JG KG
Molecular formula: $C_{12}H_8Cl_6$

Structural formula:



DIELDRIN
CAS Reg. No. EO-57-1
Toxic Substances List: IO17500
Wiswesser Line Notation: T E3 D5 C555 A D- FO KUTJ AG AG BG JG KG LG
Molecular formula: $C_{12}H_8Cl_6O$

Structural formula:



The starting materials for manufacture of aldrin (a broad spectrum insecticide) are acetylene and cyclopentadiene, which undergo a Diels-Alder condensation to form bicyclo(2.2.1)-2,5-heptadiene(1, 2), which is condensed with hexachlorocyclopentadiene to give aldrin. Epoxidation of aldrin with hydrogen peroxide catalyzed by molybdenum trioxide produces the more stable and persistent dieldrin (1). The chemical and physical properties of aldrin and dieldrin are shown in Table K-1.

Conversion of Aldrin to Dieldrin: Aldrin is readily converted to dieldrin in the environment by biological transformation. The conversion can be brought about by microorganisms (16). For example, aldrin is almost completely converted to dieldrin by the action of mushroom compost (17). Homogenates of bean and pea seedling roots were found to enzymatically oxidize aldrin to dieldrin (18). Ingestion or subcutaneous injection of aldrin into cattle, pigs, sheep, rats or poultry results in its conversion to dieldrin (19).

Four years after treatment of sandy loam, 12.6 times more dieldrin than aldrin was recovered (20). The amount of conversion was also dependent upon the type of soil (20). There was one case in which 14 days after application of 5 ppm of dieldrin to an autoclaved soil, 1 ppm of aldrin and 4 ppm of dieldrin were found by methods other than gas chromatography (21). In another study, nearly 50% of the 5.4 ppm of aldrin applied to soil in a field was lost, with a significant amount recovered as dieldrin after 21 months (21).

In three recent studies using aldrin-^{14}C at the 3 Kg/ha level on soils, roughly equivalent levels of aldrin and dieldrin were found in the soils after 6 months (22, 23, 24). Eighty percent of aldrin in river water was converted to dieldrin in 8 weeks (25). Conversion also occurs on alfalfa, soybeans and corn (26). Thus, the main loss of aldrin in the environment seems to be by conversion to dieldrin. The loss of dieldrin from soil can also occur by volatilization, run-off, leaching or photolysis.

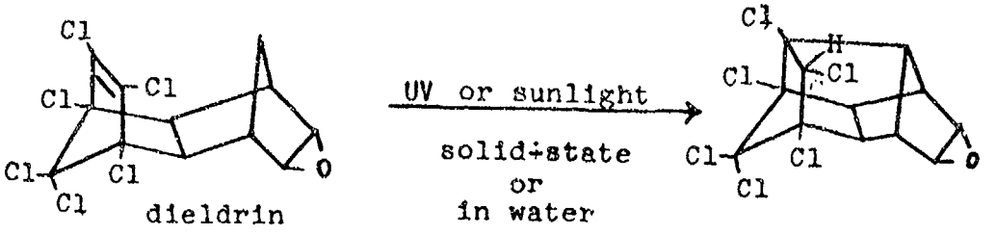
TABLE K-1. Chemical and Physical Properties

		Aldrin	Dieldrin
Melting point:	pure, °C	104-104.5° (3, 4)	175-176° (3, 4)
Vapor pressure:	torr, 20°C	7.5×10^{-5} (3)	3.1×10^{-6} (3)
	25°C	14×10^{-5} (3)	5.4×10^{-6} (3)
		6×10^{-6} (5)	7.78×10^{-6} (4)
			1.8×10^{-7} (5)
Solubility in water:		0.20 ppm (25°C) (6)	0.25 ppm (25°C) (6)
		0.39 ppm (35°C) (6)	0.54 ppm (35°C) (6)
		0.79 ppm (45°C) (6)	1.00 ppm (45°C) (6)
Solubility in organic solvents (3, 4):		0.27 ppm (25-29°C)(6a)	0.186 ppm (25-29°C) (6a)
Petroleum oils		Moderate	Slight
Acetone		Readily	Moderate
Benzene		Readily	Soluble
Xylene		Readily	Soluble
Behavior towards other chemicals (3, 4):			
Alkalies		Stable	Stable
Mild acids		Stable	Stable
Strong acids		Reacts	Fairly stable
Oxidants		Reacts	--
Cl_2		--	oxidized (7)

TABLE K-1 (Cont.)

	Aldrin	Dieldrin
4N KOH in ethylene glycol	Dehalogenates (8)	Dehalogenates (8)
BF_3/ether	--	Isomerizes to a ketone (9)
16N HNO_3	--	Stable (10)
36N H_2SO_4	--	Stable (10)
30% H_2O_2	--	Stable (10)
NH_2 CH_2CH_2OH at 140°C	Extensive dehalogenation (11)	--
Aqueous O_3	Reacts (2)	Reacts (12)
Aqueous $KMnO_4$	Reacts (2)	Stable (12)
Aqueous Cl_2	Reacts (2)	-- (12)
Response to Heat:	Stable, but slowly forms HCl on storage (3, 4)	Stable (13) DTA shows (10) endothermic peaks at 135°,175°,540° and 580°C and exothermic peaks at 325°,360° and 420°C. Complete combustion at 620°C.
Response to light:	Unstable to UV (13, 14)	Stable (3, 4, 15) instable to UV (7, 13, 14)
Adsorption on carbon (2):	6.6 ppb reduced by 90% with 100 ppm of powdered carbon - 1 hour contact	10 ppb reduced to 0.25 ppb by 30-60 ppm powdered charcoal; 4.3 and 0.5 ppb levels in water reduced to 0.05 and 0.01 ppb by granular carbon bed, respectively.

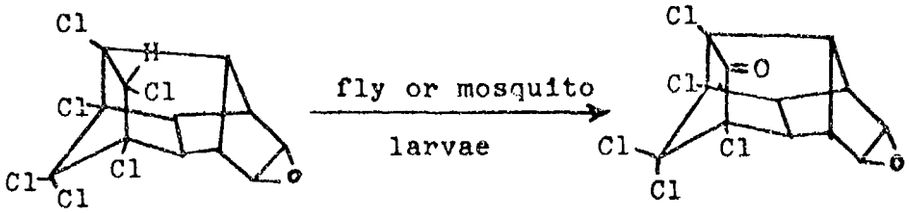
Photolysis: Photolysis of dieldrin in the solid state is known to occur on plant surfaces after several months exposure to sunlight (27). The conversion to photodieldrin also occurs in saturated aqueous solution (28, 29).



dieldrin

Photodieldrin has been found in soils in the field by electron capture gas chromatography and mass spectrometry (30). The mass spectrogram has been published (30). Out of 99 field soils, 52 samples contained dieldrin in concentrations of 1.726 to 0.002 ppm. In 14 of these 52 soils photodieldrin was present at levels 0.035 to 0.004 ppm. The ratio of photodieldrin/dieldrin ranged from 0.006 to 0.069 (30). This indicates that the amount of photodieldrin in the environment is probably not significant. Another study placed the ratio of dieldrin/photodieldrin between 16:1 to 1000:1 (31). This study also dealt with the investigation of photodieldrin in foods and in human fat. None was found in human fat; and only one food, beet foliage, contained photodieldrin at 0.02 ppm. The lower limit of detection was 0.001-0.0005 ppm. Photodieldrin is also known to arise metabolically by action of microorganisms in soil, water, rat intestines and rumen stomach contents of cows (32, 33). In addition to the photodieldrin shown above, at least three photodieldrins on grass and crops have been observed (22, 34). One of these compounds may be a chlorohydrin of photoaldrin (35).

The rate of photoconversion of dieldrin by sunlight is greatly enhanced by rotenone (36). Photodieldrin is rapidly converted to a compound known as Klein's metabolite by flies and mosquito larvae (37). Klein's metabolite was first isolated from urine of rats fed aldrin or dieldrin (38, 39).

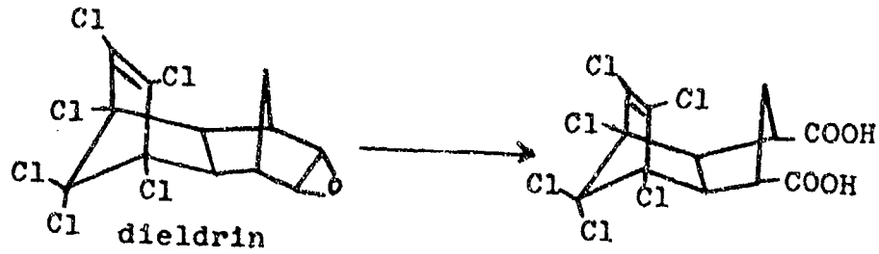


Photodieldrin

Klein's metabolite

Irradiation of aldrin with UV or sunlight yields 2 products, mp 178-9°C and mp 187-9°C (40).

In addition to photolysis, dieldrin can be converted to dihydrochlordene dicarboxylic acid (as shown below) by potatoes (24), sugar beets (23) and cabbage (41).



dieldrin

ANALYTICAL METHODS

Various gas chromatographic methods of analysis are available for the determination of aldrin and dieldrin residues in a wide variety of samples. Among the detectors available, the electron capture (EC) and microcoulometric (MC) types are most sensitive to organochlorine compounds. Of these two, the MC detector has a high degree of specificity and linearity (42).

The flame ionization detector (FID) is far more sensitive to hydrocarbons than to organochlorine compounds and is therefore not suitable to low level analysis of aldrin and dieldrin. The thermal conductivity detector (TC) is the least sensitive among the detectors discussed.

The most powerful tool in pesticide residue analysis and confirmation is the gas chromatograph coupled with a mass spectrometer (GC/MS) (43, 44). Mass spectra of aldrin and dieldrin have been published (45).

Many gas chromatographic columns can be used in the separation of pesticides and the choice of a column depends on the separation desired. Three percent OV-17 on 80/100 mesh Gas Chrom Q (46) or 4% SE-30 and 6% QF-1 on Gas Chrom Q (47) are only two of the many columns that have been used.

Table K-2 presents a list of the available references dealing with the analysis for aldrin and/or dieldrin residues in a wide variety of samples.

For positive identification of pesticides, the GC/MS system is preferred. In the absence of this capability, samples should be examined on at least two different types of columns (74). Another technique for the confirmation of residue identity is chemical derivatization.

There are a number of chemical reactions which can be used to convert aldrin and dieldrin into various derivatives (75) whose retention times relative to aldrin have been compiled. Such conversions allow positive identification of aldrin or dieldrin without the recourse to mass spectrometry (76). Some of these chemical conversions are listed in Table K-3.

TABLE K-2. Gas Chromatographic Analysis for Aldrin & Dieldrin.

Reference	Type of Sample	Cleanup	Detector	Limits of Detection
48	Plants	Channel layer chromatography	EC	0.05 ppm
49	Foods	Silicic acid-celite	--	Not set
50	Human Tissue	Extraction	EC	0.002 ppm
51	Animal Tissues	None	EC	0.1-1 ppm
43	None	None	GC/MS	0.1 µg
52	Mud, Water	--	MC	1 ppb Mud 1-10 ppt water
53	Plants Animals Soil Water	Micro column-silica gel	EC	1 ppt water 1 ppb soil, plants 4 ppb animals
44	Human Fat	Florisil	GC/MS	0.05-0.1 ppm
54	Wheat	Florisil	MC	0.1-0.5 ppm
47	Water and Sediments	Florisil	EC	10 ppt
55	Water Spiked	None	EC	1-4 ppb
56	Carrots (spiked)	None	EC	Interference
57	Lake waters (spiked)	None	EC	Limit not detn. 2 µg/l aldrin 4 µg/l dieldrin
58	Vegetables Fruits	Carbon-cellulose column	EC	Not set
59	Human Fat	Extraction-florisil	MC	Limit not set, 0.1-0.4 ppm found

TABLE K-2 (Contd.). Gas Chromatographic Analysis for Aldrin & Dieldrin.

Reference	Type of Sample	Cleanup	Detector	Limits of Detection
60	None	None	EC	4×10^{-13}g aldrin 1×10^{-12}g dieldrin
50	Various animal tissues	None	EC	0.001 ppm
61	Milk, blood, flesh of cattle, deer, geese, pheasants, fish	Extraction	EC	Not set
47	Water & sediment	Fluorisil	EC	4 ppb in sediment 10 ppt in water
62	None	None	FID	5 µg
63	Ground Water	--	--	ppb
64	Water	None	EC	ppt, not set
65	Spinach (spiked) broccoli	Florisil	MC	Not set 2 ppm detected
66	Bird Flesh, Bird Liver	Extraction	--	Not set 2 ppm detected
67	Soils	Florisil	EC	Not set 0.8 ppm detected
68	Air	None	EC	1 ng dieldrin/m^3
42	Blood	Hexane/acetone	MC	1 ng/2 ml blood
69	Plants	None	MC	Not set, at least 1 ppm
70	Surface & ground waters (spiked)	Alumina or florisil microcolumns	EC	0.022 µg/l water
71	Lake waters (spiked)	None	EC	Not set, 0.01-0.02 µg/l Detected
72	Wastewaters	TLC	EC/MC	1.5-22 ppb
73	Fish	Florisil	EC	0.01 ppm

TABLE K-3. Chemical Derivatization of Aldrin and Dieldrin for Confirmation.

Sensitivity Range	Reagent	Conversion	Ref
--	UV irradiation	Aldrin → Photoaldrin Dieldrin → Photodieldrin	77
--	BF_3/2-chloroethanol	Dieldrin → Conversion product aldrin	78
--	t-BuOCl/AcOH	Aldrin → Chloracetate	76
--	Monoperphthalic acid	Aldrin → Dieldrin	76
0.01-.1 ppm	$ZnCl_2$/HCl	Dieldrin → Characteristic compound	79
0.01-0.05 ppm	BF_3/Et_2O	Dieldrin → Ketone	80
"	HCL/ethanol	Dieldrin → Aldrin chlorohydrin	80
	Br_2	Aldrin → Dibromide	80

Prior to the advent of GC, analysis of residues by thin layer chromatography (TLC) and paper chromatography (PC) was popular. Even today these alternative methods may have some application since some methods such as TLC are rapid, simple and inexpensive and can be applied as a crude screening of samples, as illustrated in Table K-4.

In addition to these PC and TLC methods, bioassay with Drosophila melanogaster has been used for soil analysis of aldrin (94) and dieldrin (95). The eye gnat has also been used in bioassay studies (96).

TABLE K-4. Some Methods of Analysis of Aldrin and Dieldrin Other Than GC

Ref	Analytical Method	Limits of Detection Micrograms	Spray Reagent
81	PC	--	5 given
82	"	--	--
83	"	1	$AgNO_3$/UV
84	"	20	$AgNO_3/CH_2O$
85	"	0.03	$AgNO_3$/2-phenoxyethanol in acetone-UV light
86	TLC	0.5-1	o-Tolidine - UV
87	"	--	--
88	" (2-dimensions)	--	Diphenylamine/UV
89	PC	100-600	Rhodamine B/UV
90	TLC	--	--
91	"	<1	$Br_2/AgNO_3$/UV
89	"	100	Rhodamine B/UV
92	TLC (microslides)	<1	--
93	TLC	0.02-5	Fluorescence Quenching
72	"	1.5-22*	Rhodamine B

* micrograms per liter

Aldrin inhibits the enzyme hexokinase, providing a method for assay of the pesticide. The lowest detectable concentration was $10^{-6}M$ (97). Both DDT and chlordane also inhibit this enzyme.

A colorimetric method was developed for aldrin based on reaction with phenyl azide followed by reaction with dinitrophenyldiazonium salt. Aldrin spiked in cow urine was detectable at 1 ppm level with this method (98). This method has been applied to analysis of crops for aldrin and is accurate to about 0.2 ppm (99). The phenyl azide technique can be applied to dieldrin analysis (100) and levels of 0.1 ppm of dieldrin on crops have been determined (101). A colorimetric method based on the color complex formed between dieldrin and diphenylamine in the presence of $ZnCl_2$ (650 nm) has been described (102). This method is general for most chlorinated pesticides, but specificity can be gained through alkaline hydrolysis and column chromatography of the sample.

Diphenylamine has been used as a reagent for a spot test in the identification of aldrin and dieldrin. Under UV irradiation a characteristic color is produced. The sensitivity of this test is 0.2 µg (103).

Another spot test for dieldrin has been described. Dieldrin is converted to a ketone by heating with BF_3/ethyl ether complex and reacting the resulting ketone with 2,4-dinitrophenylhydrazine. The action of tetraethylammonium hydroxide on the hydrazone produces a red color; as little as 10 µg can be detected (9).

Infrared analysis of soil extracts can be used to measure aldrin and dieldrin levels. For characteristic bands, the sensitivity is 200 µg of aldrin per 0.1 absorbance unit, and 280 µg of dieldrin (104).

The Stepanov procedure has been described (105) for the determination of organochlorine compounds such as aldrin and dieldrin. It is non-specific since it is based upon the sodium and alcohol reduction of organic chloride to ionic chloride.

The extraction of aldrin and dieldrin from samples is important as the first step in the analysis of residues.

The extraction of pesticides from soil is usually carried out by exhaustive soxhlet extraction (106, 107, 108). For example, soxhlet extraction of most air-dried oils with $CHCl_3$/MeOH gives 100% recovery of dieldrin (109). Other solvent mixtures which are ineffective on dry soils give 92-98% recovery when 20% water is added prior to extraction (109). Comparison of the soxhlet method with other methods has been attempted. The use of ultrasonic energy was found to be superior to the roller and blender methods and equivalent to an 8-hour soxhlet extraction (110). In another comparison, dieldrin recovery from dry soils was the same for the ultrasonic, blender and roller methods (111).

The determination of residues in water is usually carried out by extraction with an organic solvent. Pentane is a recommended solvent for the organochlorine pesticides, but CCl_4, $CHCl_3$ and CH_2Cl_2 are not recommended (112). The partition coefficients of 131 pesticides in six solvent systems are available. The p-value for isooctane-80% acetone was 0.98 for aldrin and 0.88 for dieldrin (113). The distribution ratio between hexane and water is 10^6 for aldrin and 3.6×10^4 for dieldrin (114).

Using continuous extraction through liquid-liquid partitioning and EC-GC, ppb levels can be reached (115).

Amberlite XAD-4 macroreticular resin can be used to extract chlorinated insecticides from water at the 1 ppb level. Complete recovery of aldrin and dieldrin can be achieved (116). Porous polyurethane plugs coated with DC-200 oil will efficiently extract aldrin and dieldrin from water at the 1-4 ppb levels (55). The advantage of the Amberlite and polyurethane plug methods is in their ability to extract organochlorine compounds from large volumes of water.

MAMMALIAN TOXICOLOGY

Human Exposure

Accidental (or intentional) Ingestion or Dermal Exposure: Up until 1955, world medical literature reported 13 fatal cases of aldrin-dieldrin poisoning (suicide attempts, industrial accidents, spraying mishaps, etc.) (125). From various considerations, the lethal dose of aldrin/dieldrin for an adult man is estimated to be about 5 grams (118).

Occupational Exposure: The most comprehensive single study of aldrin/dieldrin in occupationally exposed men is on 233 workers engaged in the manufacture of aldrin or dieldrin (as well as the related compounds, endrin and telodrin) who had been exposed for at least four years (and up to 13 years) (125). The entire period of study covered 15 years. In numerous cases of accidental intoxication among the plant workers, there were no fatalities. Toxic signs noted were confined to the central nervous system and were reversible. No evidence of liver disease was seen in individuals after long-term exposure (4-13 years), and no unexpected changes in hemograms, blood enzyme patterns, serum proteins, etc., were observed. No central nervous system or renal disturbances were seen. In this group of 233 men, the mean blood level of dieldrin was 0.035 µg/ml, corresponding to an approximate average daily oral intake of 407 µg/man/day (119). This is about 50 times the daily intake of the general United States population (vide infra). Moreover, in the 32 workers with exposures of over 12 years, the mean dieldrin blood level was 0.008 µg/ml, with a top figure of 0.06 µg/ml. The threshold blood level below which signs of intoxication do not occur was felt to be 0.2 µg/ml for dieldrin--a value said to be in agreement with other medical literature. Only the serum glutamic-oxalacetic and glutamic-pyruvic transaminases showed slight increases with increasing dieldrin-equivalent* blood levels. These values were still within normal limits.

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*Dieldrin plus aldrin plus telodrin plus endrin, calculated as dieldrin gives dieldrin equivalent.

The half-life of blood dieldrin was computed to be 0.73 years in the occupational workers study (125) compared to a calculated value of 1.01 years in an experiment with human volunteers (119). Workers with blood levels of dieldrin about 175 times that of the general population average showed no effects on p,p'-DDE metabolism. Since known enzyme inducers (diphenylhydantoin, phenobarbital) reduce p,p'-DDE levels in blood, this is considered evidence that aldrin/dieldrin did not stimulate hepatic microsomal induction in these workers (125). But endrin workers did show this effect (125). Others with blood dieldrin levels about 85 times that of the general population showed no changes in urinary corticosteroid excretion ratios.

Controlled Feeding Studies: Controlled feeding studies in human volunteers have followed the subjects for two years (119, 120). Daily dose levels (given in olive oil in gelatin capsules) were 10, 50, and 211 µg of dieldrin per day; a control (zero dieldrin) group received capsules only (plus whatever dieldrin was present in the diet). Besides extensive clinical chemistry evaluations at regular intervals, blood and adipose tissue analyses for dieldrin were made. The general conclusions of this study were: there were no abnormalities produced; an equilibrium in body burden of dieldrin was reached in 9-12 months; a mathematical relationship existed between dieldrin concentrations in blood and fat and the daily intake at equilibrium. Upper levels of dieldrin were 0.02 µg/ml in blood and 2.85 µg/g in fat of subjects that received 211 µg/day. Since it was estimated that the dietary intake was another 14 µg/day, the top dose level was thought to be 225 µg dieldrin per day. As mentioned above, the half-life of dieldrin in these subjects was calculated at 1.01 years (369 days).

In connection with hepatic microsomal enzyme induction, Robinson (121) has stated, "In the case of volunteers who had ingested up to 230 µg HEOD* per day (0.003 mg/kg body weight per day), and whose body burden ... was about 10 times that of the average person in the U.K. or U.S.A., the concentrations of o,p'-DDE in the whole blood did not decrease. It was tentatively concluded that the rate of metabolism of p,p'-DDE in man was not increased by a ten-fold change in body burden of HEOD...."

Since organochlorine insecticides, including aldrin-dieldrin are stored chiefly in fat, the question arises of sudden, massive release of these toxic materials owing to abrupt metabolism of fat deposits (as during high fever, severe reducing diets or after major surgery). No significant elevation in blood dieldrin levels or toxic signs is observed in patients following elective surgery (122).

Exposure of General Population: It has been estimated that 90% of the total intake of organochlorine insecticides by the general population in the U.S. is from food residues (125). Human dietary intake has been extensively studied and summarized (123):

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* Dieldrin contains at least 85% hexachlorepoxy-octahydrodimethanonaphthalene (HEOD)

	Year						6-Year
	1965	1966	1967	1968	1969	1970	Average
	µg/kg/day						
A+D	0.09	0.13	0.06	0.06	0.07	0.07	0.08

The 6-year average corresponds to 5.6 µg/day for a 70-kg individual. (The acceptable daily intake - ADI - set by the WHO/FAO is 7 µg/70-kg person/day).

In this regard attention may also be drawn to the drinking water limits that will probably be imposed by the EPA, as discussed in the Federal Register of March 14, 1975 (124). These maximum limits are likely to be 0.001 mg/l each of aldrin and dieldrin.

Analysis of human tissues has shown the presence of dieldrin (50). Table K-5 shows the distribution found.

TABLE K-5. Average Levels of Dieldrin in Human Tissues Analyses in Duplicate and Triplicate From Four Persons (50).

Organ	Dieldrin ppm
Kidney	0.007
Brain	0.008
Liver	0.015
Gonads	0.024
Fat	0.068

In another study the pooled fat from 10 people was analyzed and dieldrin found at the level of 0.4 ppm (31). No photodieldrin was found in the fat.

Another investigator found between 0.1 and 0.4 ppm dieldrin in 45% of the human fat samples studies. Fifty-five percent of the samples were free of dieldrin (59).

Further information on human tissue dieldrin levels relates mostly to fatty tissue, and through 1970 the mean concentrations ranged from 0.12 to 0.15 ppm (125). The body fat of stillborn infants and fetuses has

been found to run 0.13-0.24 ppm (126) (pg. 369). While adult adipose tissue levels largely result from food intake, those in neonates and fetuses must arise from placental transfer. Parenthetically, human breast milk concentrations have been reported in the range of 4.5-7.3 ppb(126). Relatively elevated body fat levels of dieldrin (0.37-0.73 ppm) have been noted in hospitalized individuals dying of carcinoma, atherosclerosis and hypertension (127). These increases are believed to be the result of the general inanition seen in such terminal patients. The insecticide level in other tissues increases in concentration as the total fat decreases from the wasting process of the disease.

Studies on the distribution of aldrin/dieldrin in the blood of six formulators working with aldrin for five weeks indicate dieldrin was in greater concentration than aldrin in all components examined (128). The plasma to erythrocyte ratio of dieldrin averaged 3.77 to 1, and the α- and β-lipoprotein fractions carried much of both compounds.

Feces of men occupationally exposed to dieldrin contain 9-hydroxy-dieldrin (129). The presence of at least two neutral polar, chlorinated metabolites of dieldrin has been detected in urine of such workers.

Three areas in human toxicology (or potential toxicology) remain to be mentioned: teratogenicity, carcinogenicity, and mutagenicity. The remarks in Deichmann, 1973, (126), with references given therein, serve present purposes very well. Thus:

> "From the present state of knowledge, it must be agreed that no firm conclusions can be drawn as to whether pesticides represent a mutagenic hazard to man. The area of mutagenicity of testing is in its infancy, and much more experimental investigation is needed." (pg. 312)
>
> "Teratogenic effects of pesticides would have to be obtained from epidemiologic data...there appears to be no conclusive evidence that the small number of pesticides which have been studied for teratogenic potential actually represent a hazard to humans under normal conditions of pesticide exposure .... There is no good epidemiological evidence implicating pesticides in teratological toxicity in man." (pg. 318)
>
> "A causal relationship between a particular organochlorine pesticide and human cancer has not been documented." (pg. 402)

and finally,

> "... even if a positive relationship between tissue pesticide content and occurrence of cancer were shown, an epidemiological relationship will still not be established. One would still not know which factor (pesticide storage level or cancer) was cause and which was effect or whether both were effects of a third factor. Also, the effects of emaciation which usually occurs in fatal cancer must be evaluated." (pg. 329)

O'Brien et al. (130) concluded:

> ..."one cannot dismiss the possibility that sufficient doses of dieldrin could be carcinogenic in man, but the degree of certainty is inadequate to require prompt elimination from the diet. One must also note that in rats, dieldrin at 20 ppm or more in the diet decreased malignant mammary and lymphatic tumors; for instance, in males 14% of controls had such tumors, but only 6% of treated animals had them."
>
> "It seems clear that the species selected for test is important. Furthermore, there is reason to believe that fetal organisms, because of their high mitotic rate, might be unusually sensitive and it is clear that placental transfer occurs in humans. Consequently, we would like to see studies on effects upon fetal animals whose mothers are treated and which are also postnatally exposed."

In conclusions on human toxicology of aldrin/dieldrin, it is worth noting that of the 233 workers with long-term (4-13 years) exposure to these cyclodiene compounds only three malignant tumors were detected (125). This cancer incidence was not interpreted as being significantly different from that of a control population.

Experimental Animals

Acute and chronic: Information on acute chronic oral toxicity of aldrin/dieldrin has been summarized (118), and much early (but still valid) data are to be found in Volume 3 of Handbook of Toxiciology (131), with references to original literature.

Acutely, the oral toxicity (LD_{50}) of aldrin has been reported from about 20 to 120 mg/kg for 12 mammalian species. For dieldrin the range is about the same, although the figure for the cat has been reported as about 400 mg/kg (118). Variations in the vehicle used in various studies and in concentrations used influence the LD_{50} obtained, and young individuals of a species may, in general, be more susceptible than older ones. Effects of a single oral dose of aldrin/dieldrin are long lasting, and may persist for up to three weeks.

These compounds are readily absorbed through the skin irrespective of the type of formulation in which they are incorporated. Acute dermal LD_{50}'s vary even more widely than values for oral doses. For the rabbit, a "standard" species for dermal studies, the LD_{50}'s of aldrin and dieldrin have been quoted as 320 and 560 mg/kg, respectively (Hodge, 1967) (118); other figures are as low as < 150 mg/kg for single application of dry compound, and as low as 10-50 mg/kg for repeated daily dermal exposures (131).

Repeated oral administration of aldrin/dieldrin over relatively short time periods produces no mortality at less than 25 parts per million (ppm)

in the diet. In chronic studies (1-2 years), however, mortality is seen at 10-20 ppm, and monkeys fed 5 ppm died in one year. Other monkeys tolerated 1 ppm (118). Several studies have suggested that starved rat, or those on low protein diets, were more susceptible to dietary dieldrin (118).

Tissue and organ response: Growth and body weight are not especially sensitive indices of aldrin/dieldrin feeding effects. However, increased liver-to-body weight ratios have been seen in rats given 0.5 ppm of aldrin/dieldrin and in dogs given 3 ppm. In the liver of rats, the characteristic "chlorinated insecticide" lesion is seen, and this consists of "enlarged centrilobular hepatic cells, with cytoplasmic oxyphilia...and peripheral migration of the basophilic granules" (132). The same authors indicated hepatic cell changes were seen in dogs given 0.5 mg/kg/day of dieldrin for up to 81 weeks.

In another two-year feeding study rats at dietary levels of 0.1, 1.0, and 10 ppm showed nervousness and irritability at 10 ppm, and liver-to-body weight changes at 1.0 ppm and above, but liver histopathology only at 10 ppm. Other parameters of the study (hematology, clinical chemistry) were not affected. A single dose of 3 mg/kg aldrin lowered conditioned reflexes in cats, but 1 mg/kg had no effect. Daily doses of 1 mg/kg for 8 to 13 days changed the conditioned reflex,thus there seems to be a cumulative effect; in aerosol, 0.0001 mg/liter for 4 hours daily was sufficient to cause changes in conditioned reflexes (134).

Dogs fed the equivalent of 0.1 and 1.0 ppm of dieldrin for two years had liver-body weight increases in females fed 1 ppm, but no histopathology (133). Of the other parameters studied, only serum alkaline phosphatase was elevated at 1 ppm. No tumors were seen in the rats or dogs in these long-term studies.

A chronic toxicity study in the rhesus monkey, in progress since 1963, has shown (after 5-6 years) no microscopic, electromicroscopic, or chemical evidence of liver changes that could be attributed to dieldrin (125).

Carcinogenicity: With respect to carcinogenicity in laboratory animals, results with the rat and the dog studies have failed to show that aldrin/dieldrin are tumorigenic or carcinogenic in these species (133). A chronic rat feeding study also failed to show tumorigenic effects at dietary levels as high as 50 ppm (135). However, the hepatocarcinogenicity of aldrin/dieldrin for the mouse, or at least for certain strains of inbred mice, has been demonstrated (136, 137, 138). Experimental evidence indicates that 10 to 50 ppm of aldrin and 5 to 10 ppm of dieldrin are toxic to mice (139, 140). Tumors were noted in mice given 0.5 and 2.0 ppm aldrin and dieldrin, however, the carcinogenicity is questioned since the rates for 0.5 and 2.0 ppm were the same (140).

About various studies with various species, the Working Group of the International Agency for Research on Cancer has commented, "...the

hepatocarcinogenicity of dieldrin in the mouse has been demonstrated and confirmed in several experiments, and some of the liver-cell tumors were found to metastasize. A dose-response effect has been demonstrated in both sexes with an increased tumor incidence in females at the lowest dose tested, 0.1 ppm in the diet (corresponding to above 0.015 mg/kg day).... The available data in rats have not provided evidence of carcinogenicity at levels up to 50 ppm in the diet (corresponding to an intake of about 2.5 mg/kg bw/day).... The experiments in dogs and monkeys were too limited in duration and/or group sizes to allow any conclusions to be made (141).

The National Cancer Institute (142) has aldrin undergoing carcinogenesis bioassay in the carcinogenesis program of the Division of Cancer Cause and Prevention. The pesticide is being fed to mice (strain B6C3F1) and to rats (strain OM).

Teratogenicity: No evidence of teratogenic effects were seen in the rats or dogs in the studies above, but it is reported that oral doses of aldr / dieldrin or endrin to pregnant golden hamsters produced fetal deaths and congenital anomalies (cleft palate, webbed feet, open eyes), often in combination (143). Mice treated in this same study showed these terata, but not the fetal mortality.

Reproductive effects: In mice 5 ppm of dieldrin fed for 120 days reduced the size of all litters (144). A three-generation study in rats (118) showed that the lowest level of aldrin to have an effect on rat reproduction was 12.5 ppm, while only 2.5 ppm of dieldrin adversely affected the rat. Dieldrin administered to male mice caused reduction in assimilation of androgens and altered metabolism of teststerone (145). Dog reproductive data are limited; 8 ppm of aldrin and 25 ppm of dieldrin are reported to have increased pup mortality (118).

From the standpoint of environmental impact, an experiment where dogs were not bred until two weeks to 16 months after discontinuation of aldrin feeding, and the blood and fat levels of dieldrin were comparable to those found in pesticide operators and volunteers, has serious implications (146). The delayed estrus periods seen in the females, reduced pup survival rates, elevated stillbirth rates and severely depressed lactation performance all indicated subnormal reproductive processes. The findings are consonant with the well-known effects of the chlorinated insecticides on liver microsomal enzymes responsible for steroid metabolism, and suggest that reproductive derangements can occur long after the exposure to pesticides has stopped (147). The implications for mammalian wildlife existing on a narrow margin of survival are plain.

The placental barrier is crossed by dieldrin, which concentrates in the fetus, and lactating animals excrete dieldrin in the milk (148).

Storage and excretion: Aldrin is converted to the epoxide, dieldrin, by the liver microsomes (149) and the enzymes responsible for this epoxidation are inducible (150). After oral administration dieldrin is absorbed from the upper gastrointestinal tract and passes to the liver, kidneys, and major fat depots. Thereafter, redistribution occurs, with organ levels stabilizing and fat levels increasing to plateau levels when dietary intake continues. The length of time required to reach plateau storage levels is a function of intake levels (151).

Biliary excretion occurs, about 90% of a single dose being excreted in the faces as hydrophilic metabolites, and 10% in the urine (125). Different species metabolize dieldrin differently, and 9-hydroxy HEOD, 6,7-transdihydroaldrindiol, and a "pentachloroketone" are among the metabolites identified (152). The transdiol, at least, is said to be much less toxic than the parent material, with an oral LD_{50} in the mouse of 1250 mg/kg (130).

The transdihydroaldrindiol and 9-hydroxydieldrin were also found in sheep urine as well as other unidentified materials (153). Biochemical work has indicated dieldrin (acutely) inhibits glutamine synthesis in brain and allows buildup of ammonia, which in turn contributes to the convulsions seen during acute dieldrin poisoning (154). Other phases of dieldrin metabolism, as blood-brain barrier passage, gut wall passage, placental transfer, and blood components partitioning are summarized in this article.

A review of the metabolism of cyclodiene insecticides is given by Brooks (155), covering literature through June 1968.

Acutely, photodieldrin is more toxic (about five-fold) than dieldrin to rats, mice, guinea pigs and pigeons, but in the dog it is similar in toxicity. Subacutely, mice were more susceptible to photodieldrin than to dieldrin (139); but the rat tolerated photodieldrin about as well as the parent material (156).

Unpublished work has shown that rats excrete the same keto-compound in the urine from photodieldrin as from dieldrin (121).

Mutagenicity: Aldrin and dieldrin do not show any mutagenic activity when tested in Drosophila melanogaster, using the Muller-5 genetic test (157). No evidence of mutagenic hazard from dieldrin was found using dominant lethal and host-mediated assays in mice (157). Moreover, dieldrin manufacturing workers showed no significantly different degree of chromosome damage than did control workers (157).

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Transport: Prevalence in soil.--- The occurrence of dieldrin and aldrin in the environment is widespread. Ontario farms contained aldrin and dieldrin in amounts in excess of 0.1 ppm in 16 out of 31 soils examined (158). In sandy loam, aldrin and dieldrin can persist for up to 15 years yielding 40% and 31% recovery, respectively (159). Mucky soil retains aldrin better than silt loam (159). Aldrin and dieldrin persist despite flooding (160).

Leaching.--- Penetration of aldrin and dieldrin into soil occurs slowly (161). Of total aldrin applied to 5" of soil, only 23% had moved to the 6 to 9" depth after 15 years (162). After 17 months, 90% of applied aldrin

(200/lb/acre) still remained in the top 3 inches of a loam soil; aldrin and dieldrin dissipate at approximately 30% a year primarily due to microbial and light degradation of the compounds and vaporization of the compounds (163). Estimates have been made that "several hundred years" would be required to transport 20 ppm dieldrin in H_2O 12" down into the soil from residues near the soil surface (164). Therefore little danger is presented by aldrin or dieldrin entering the ground water (161).

Other investigators have calculated a leaching index of <10 cm for both aldrin and dieldrin dispersal through the soil profile with an annual rainfall of 150 cm (165). These short term studies were made in four countries using a 3kg/ha application of aldrin-^{14}C to field soils. Table K-6 presents the levels of aldrin and dieldrin in the soil profile after a 6-month period (22, 23, 24).

TABLE K-6. Soil Profiles in Four Countries Obtained During Three Separate Studies (22, 23, 24).

Location		Soil Depth (cm) 0-10	10-20	20-40	40-60	Rainfall (cm) During Period
		Residue in ppm				
Germany	Aldrin	0.78	0.18	0.03	<0.01	55.4
	Dieldrin	0.55	0.16	0.04	0.01	
	Aldrin	0.58	0.23	0.02	<0.01	55.4
	Dieldrin	0.62	0.26	0.02	<0.01	
	Aldrin	0.87	0.38	0.08	0.01	55.4
	Dieldrin	0.68	0.28	0.07	0.01	
England	Aldrin	1.30	<0.01	<0.01	<0.01	29.0
	Dieldrin	0.72	<0.01	<0.01	<0.01	
	Aldrin	0.59	<0.01	<0.01	<0.01	--
	Dieldrin	0.40	<0.01	<0.01	<0.01	
U.S.	Aldrin	0.50	0.01	<0.01	<0.01	Not detm.
	Dieldrin	1.17	0.01	<0.01	<0.01	
Spain	Aldrin	0.83	0.02	0.01	<0.01	Not detm.
	Dieldrin	0.60	0.04	0.02	0.01	

These differences in the disparities between the concentrations at the 0 to 10 cm and lower depths in the different countries probably result from differences in soil permeability (soil type). Although it appears that dieldrin itself does not leach readily, a metabolite of dieldrin, dihydrochlordenedicarboxylic acid, was detected in the leaching water draining from an experimental box at a depth of 60 cm (24).

Lateral seepage also occurs. A field with a 5 to 15% slope showed 1.3 to 2.2 times more applied aldrin on the lower than on the upper half (161). Dieldrin binds to soils, as montmorillonite clay forms a complex with dieldrin which is tan in color and exhibits an ESR signal (166). In a comparison of changes in dieldrin concentration in a soil profile over a 4-year period, three plots with different initial levels of dieldrin were studied (167). The data are presented in Table K-7.

TABLE K-7. Quantities (in ppm) of Dieldrin in Light Sandy Soil Compared with Amounts Present 4 Years Previously (167).

Compound	Plot	Year	Residues in ppm (µg/g) Soil layer in cm					
			0-10	10-20	20-30	30-40	40-50	50-60
Dieldrin	B	1969	1.25	0.23	0.02	0.01	...	...
		1973	0.77	0.71	0.17	0.03	0.02	0.04
	C	1969	2.29	0.86	0.02	0.01	...	...
		1973	1.73	1.72	0.33	0.04	0.02	0.02
	D	1969	7.33	2.50	0.05	0.03	0.02	0.01
		1973	7.3	8.0	1.3	0.25	0.10	0.11

These data seem to indicate that not only is there some downward leaching, but the proportion that leaches to the 10 to 30 cm depth, persists longer.

Run-Off.---A 1972 EPA study points to loss of aldrin and dieldrin by means of sediment run-off as the dominant route for water contamination. Millions of tons of sediment are carried in this country's major rivers; and from different bases, as little as 260 pounds or as much as 14 tons of dieldrin are annually carried in the Mississippi past St. Louis. In this report, Iowa farm soils averaged 107 ppb and Iowa river sediments averaged 11 ppb (168).

Prevalence in Water. --- In water dieldrin seems to persist for considerable time. Dieldrin is common in Scottish waters where some of the cleanest streams contain 0.01 µg dieldrin/liter, a level considered harmful to wildlife (169). In one study, dieldrin placed into river water underwent no change after 8 weeks (25). The level of dieldrin in U.S. surface waters has been estimated to be about 0.4 ppb (28). Analysis of mud and water has revealed aldrin at levels of 1-10 ppb, respectively (52). The ratio of concentration of insecticides in sediment to concentration in water ranges from about 10 to 10,000 ppm (47, 161). In a study on North Carolina's bays and rivers, dieldrin was detected in only one out of 154 water samples gathered over a 13-month period. During this period, 150 sediment samples were collected and only 6 contained dieldrin (47). The highest dieldrin concentration found in sediment was 18 ppb (47). A study of South Carolina's ground waters showed aldrin levels of 0.007 ppb (170). These data show dieldrin residues are most likely found in soil rather than water.

Volatilization. --- Volatilization from soil may be the main path in the loss of aldrin and dieldrin from soil (171). However, when a compound is bound to the soil surface, the vapor pressure cannot be used directly as an index for vapor transport (165). A vaporization index has been given for aldrin and dieldrin in soil as less than 0.1 kg/ha/year (165). The vapor density of dieldrin - soil mixtures was found to be 54, 202 and 676 ng/l at 20°, 30°, and 40° respectively (172). The vapor density of dieldrin in dieldrin-soil mixtures increased with temperature, but was not affected by water content of the soil until the content decreased to below that equivalent to 1 molecular layer of water. Below this water content, the vapor density dropped to very low values, but increased again as water was added, indicating that the drying effect was reversible (173). Field measurements were made of the volatilization of dieldrin from soil which had been sprayed with dieldrin emulsion at a rate of 5.6 kg/ha and immediately disked into the soil to a depth of 7.6 cm. Dieldrin was shown to be present in the air during 18 weeks at heights of 30, 45, and 60 cm above the surface. The collected dieldrin represented 2.9% of applied pesticide (174).

Potential volatilization rates have also been reported. These are presented in Table K-8 and are based on the rate of loss in the initial 24-hour period. These figures represent the maximum volatilization rates likely to occur under optimal conditions (174).

In summary, the volatility of dieldrin is influenced by the water content of the soil, air flow (wind), temperature, and concentration in soil.

The soil concentrations of aldrin and dieldrin decrease through time by leaching, runoff, volatilization and degradation. The half-life of aldrin and dieldrin in soils has been estimated to be 4 and 7 years, respectively (175).

TABLE K-8. Potential Volatilization of Dieldrin From Gila Silt Loam at 10% Water Content and 100% RH (174).

Soil conc. (ppm)	Air flow (miles/hr)	Volatility (kg/ha/year) Dieldrin at 20°C	Volatility (kg/ha/year) Dieldrin at 30°C
1	0.005	0.24	0.69
	0.018	0.39	1.4
5	0.005	1.8	3.8
	0.018	2.4	8.9
10	0.005	2.6	8.7
	0.018	5.4	14.2
50	0.005	3.7	15.2
	0.018	7.5	21.9

Animals

Mammals: Information on mammals is in the section on "HUMAN TOXICOLOGY", under *"Experimental Animals"*.

Birds: As little as 1 ppm of aldrin in feed on a continuing basis can lead to high mortality in quail (176). In pheasants, 5 ppm in feed leads to low hatchability and other reproductive complications (177). Dieldrin is not as toxic to quail as aldrin; 1 ppm in the diet over time (in winter) can be tolerated (176). Pheasants and pigeons can suffer adverse effects with 5 ppm dieldrin in their diet (176).

The following is an extract of a report by O'Brien et al., 1972 (130), regarding birds (the authors' numerical designators of references have been left out of the text):

> "The acute oral LD_{50}'s for aldrin for four avian species are 6.6 mg/kg in the female bobwhite quail, 16.8 mg/kg in the female pheasant, 29.2 mg/kg in the male fulvous tree duck and 520 mg/kg in the female mallard duck. The highest daily dose that can be tolerated for 30 days by the mallard is 5 mg/kg. The symptoms of poisoning by aldrin in birds include ataxia, circling, low carriage, closure of the nictitating membrane, tremors, phonation, wing-beat convulsions, seizures and opisthotonos. Death occurs from 1/2 hour to 10 days after treatment."

"Feeding studies in birds with aldrin indicate a no-effect level of about 1 ppm. One-day old quail on a diet containing 1 ppm aldrin survived for 47 days, one-day old pheasants started on a diet containing 5 ppm aldrin exhibited 100% mortality by the 46th day. Symptoms of poisoning at these levels occurred 48 to 72 hours after the initiation of treatment; the symptoms are those that are seen in adult birds with acute poisoning. Five ppm aldrin will cause 100% mortality in adult quail and pheasants."

"The effects of aldrin on reproduction in birds indicate a decrease in egg production with a level of 1 ppm, with a cessation of egg production by the sixth week. The hatchability of the eggs laid by birds fed 10 ppm decreased as did the fertility. There was no effect on chick viability at this level."

"The acute oral toxicity of dieldrin has been determined in various domestic and wild species of birds. In the chicken, the oral LD_{50} for adults has been reported to be between 20 and 30 mg/kg while other studies indicate that 44 mg/kg causes no mortality. In wild species the LD_{50} is reported as being 381, 79, 23, 70, 27, 48, and 9 mg/kg for the mallard, pheasant, chukar, coturnix, pigeon, sparrow and gray partridge, respectively. The acute dose for the Canada goose is between 50 and 150 mg/kg. The daily dose that can be tolerated for 30 days is 2.5, 1.25 and 5.0 mg/kg for the fulvous tree duck, gray partridge and mallard, respectively. The symptoms of acute poisoning are hyperexcitability, jerky gait, ataxia, dyspnea, myasthenia, fluffed feathers, immobility, opisthotonos and terminal wing-beat convulsions. Death occurs within 1 to 9 days after poisoning."

"In feeding studies, the administration of 5 ppm dieldrin to day-old quail causes 100% mortality, while 0.5 ppm has no effect on survival. One ppm causes 100% mortality after 76 days. A level of 5 ppm will cause 100% mortality in pheasants by the 68th day. The susceptibility of adult birds to repeated feeding of dieldrin is not as great. Adult pheasants fed 100 ppm dieldrin exhibit 100% mortality between 10 days in the males and 39 days in the females. In quail, 10 ppm dieldrin has no effect while a level of 20 ppm causes 50% mortality between 13 and 63 days."

"Ten ppm dieldrin fed to quail causes a decrease in the hatchability of eggs and the survival of chicks. Other reports indicate that levels of 20 to 30 ppm are needed to cause a decrease in egg laying. At the 20 and 30 ppm levels there is increased chick mortality by the 3rd day after hatching. In pheasants there is a slight decrease in egg laying by birds fed 25 ppm, while 50 ppm significantly decreases egg laying. The survival of the chicks from the eggs of female pheasants fed 50 ppm is decreased by 35%. In the gray partridge, 3 ppm dieldrin given as a pellet did not affect fertility or egg hatchability, however, there was a slight increase in mortality in the shell. The growth

rate and chick survival after hatching were not affected by this level. Dieldrin at levels of 1.6, 4 and 10 ppm given to penned mallards caused a decrease in eggshell thickness."

"The population of wild birds in areas treated with dieldrin did not change after dieldrin application. The clutch size and hatchability of gallinules are not affected when eggs contain as much as 13 ppm dieldrin. The use of rice bran contaminated with residue levels of dieldrin to feed leghorn hens had no effect on egg production, hatchability or chick survival. There is a correlation between the amount of dieldrin found in the eggs and the amount of dieldrin fed to the birds. A dietary level of 20 ppm fed to quail can cause over 45 ppm to be found in the eggs after 7 weeks."

The hazards are less extreme than to fish (130):

"Thus rather high levels of dieldrin (ca. 1 ppm in the diet) are needed for production of thin-shelled eggs in ducks, in one of the few carefully controlled experiments. In sparrow hawks, the high dose of 3 ppm of dieldrin plus 15 ppm of DDT produced a maximum eggshell thinning of 16%. Furthermore, the reported effects of dieldrin upon carbonic anhydrase (thought to be causal in eggshell thinning) have recently been shown to be artefactual, being caused by coprecipitation of the soluble form of the enzyme used in laboratory studies. Much of the data on effects upon wild birds is impossible to evaluate because dead birds were collected without establishing the cause of death, and they contained a variety of pesticide residues. Data upon bald eagle deaths is particularly suspect in this regard."

"Nevertheless, we accept that in at least some avian species, quite low levels of aldrin or dieldrin may have adverse effects; thus 1 ppm of aldrin in the diet reduced egg production of pheasants by 17%, in quail by 23% and 1 ppm of aldrin or dieldrin was lethal to 100% of quail chicks. To avoid adverse effects on wildlife, aldrin and dieldrin must be used in ways which cannot lead to intake levels of several ppm for birds. The use of aldrin applied directly to soil at 1 lb/acre over 16 years leads to levels of about 1 ppm (aldrin plus dieldrin) in a variety of insects, and less than 0.02 ppm in a variety of seeds of plants grown in that soil. Such usage is unlikely to lead to substantial effects on wildlife."

Dieldrin at 4 ppm fed to mallard ducks decreases the hatchability of fertile eggs by 50%, but this is not due to eggshell thinning (178). The amount of eggshell thinning from dieldrin differs for different species

of water birds, but is generally higher with higher organochlorine residues (179, 180).

Aldrin and dieldrin have been shown to accumulate in pheasants under semi-natural conditions with little or no effect on mortality or weight gain (181). Dieldrin is known to accumulate in Japanese Quail (182) and may lead to increased accumulation of DDE residues (183).

Aldrin and dieldrin are known to accumulate in Golden Eagles of the United States but do not cause acute toxic effects (184). Dieldrin causes internal organ size changes in pigeons (185).

Egg production in domestic fowl fed 20 to 200 ppm dieldrin appears to increase (186, 187). However, these levels of dieldrin may also increase chick mortality (187) and affect egg shell thickness from clutch to clutch (186).

Fish: Both aldrin and dieldrin are extremely toxic to fish. The median tolerance limits (TLm) which are equivalent to the LD_{50} for a specified exposure period has been reported to be 0.0155, 0.012, 0.0075, and 0.067 ppm for periods of 24, 48, 72, and 96 hours, respectively, for the pumpkinseed sunfish. In a 96-hour exposure period, levels of 0.32, 0.0155, 0.0087, and 0.0075 ppm were highly toxic while 0.0056 ppm caused no mortality. However, the level of 0.0056 ppm caused 100% mortality by the end of one week. The 96-hour TLm for minnows, bluegills, goldfish and guppies is between 0.015 and 0.037 ppm. Exposure of steelhead trout to 1.2 ppb for 45 days leads to 100% mortality (130). Carp had a 48-hour LD_{50} at 6.7 ppb (188) and 6 ppb was toxic to green sunfish after 124 hours (189). Twelve ppb killed all exposed sailfin mollies, Poecilia latipinna, within 72 hours (190).

Levels of 0.0056 and 0.0032 ppm of dieldrin had toxic effects in the pumpkinseed sunfish by decreasing cruising speed and increasing the consumption of dissolved oxygen. Difficulty in orienting to the current and an increased sensitivity to sunlight were also noted. A concentration of 0.00168 ppm of dieldrin increased oxygen consumption and decreased cruising speed in sunfish (130). Feeding dieldrin 140 days to rainbow trout altered the normal concentration of 11 amino acids (191).

Guppies exposed to 0.01 ppm dieldrin produced no fry after the 32nd week of exposure. In this study, the authors noted an initial increase in the population which they attributed to less predation of the young by the adults. Exposure of steelhead trout to a level of 0.39 ppb dieldrin resulted in only a 3% survival rate of fry to the age of 130 days. The growth of trout was not affected by levels of 0.12 ppb and below (130).

The 24-hour LC_{50} for dieldrin to brown trout (Salmo trutta Linn.) was 0.016 ppm, and minimum lethal levels in tissue were between 1-2 ppm, according to Dacre and Scott, 1973 (192).

Concerning the toxicity of aldrin/dieldrin to fish, O'Brien et al. (1972) concluded (130):

> "Data upon the toxicity of aldrin and dieldrin to fish and crustaceans lead us to accept such terrible accounts as that describing the effects of 1 lb/acre of dieldrin on 2000 acres of Florida salt marsh used for sandfly control: "... fish kill was substantially complete. The minimum immediate overall kill ... was 20-30 tons of fishes.... Crustaceans were virtually exterminated throughout the area" (193). For such reasons, we believe that applications to aquatic habitats must be forbidden; one should recall that even 3 ppb in water can cause measurable toxic effects in some fish."

Snakes and Turtles.--- Residues of dieldrin are found in snakes, with highest residues associated with water snakes (194). Dieldrin readily accumulates in fat tissue of turtles (195).

Invertebrates: Crawfish.--- The five-day median tolerance limits (TLm) for aldrin in Louisiana red crawfish (Procrambarus clarkii) was reported to be 56 ppb. However, 200 times this concentration in the soil had no effect on survival or growth of the crawfish (130). Other studies of this crawfish species have reported aldrin TLm's of 38 ppb for juveniles and 600 ppb for adults (196). Adult crawfish were not affected by treatment of rice seed at rates of 0.25 and 0.5 lbs aldrin per 100 lbs of seed (196).

Oyster.--- Oysters exposed for 10 hours to water containing 1 ppm dieldrin exhibit physiological irritation manifested by a continual opening and closing of the valves, indicating an abnormal feeding process. After two weeks of exposure to 0.1 ppm dieldrin the oysters were only half as active as the controls. There is interference of shell deposition by the oyster in the presence of dieldrin. Oysters also store chlorinated hydrocarbons present in ambient concentrations of 0.1 ppb or more (O'Brien et al., 1972) (130).

Earthworms.--- Applications of aldrin at 4.7 kg/ha had little effect on earthworms, while dieldrin at 5.6 kg/ha was lethal (197). Dieldrin at 46 g/100 sq. in. reduces the earthworm population from 240 to 2 after 2 years (198). In soils in which aldrin/dieldrin residues ranged 0.0029 to 0.083 ppm (dry weight), residues in earthworms were 0.074 to 0.78 ppm (199). Treatment of soil with 1.25% aldrin dust (at 300 ml/acre) did not affect earthworms (200). Laboratory studies indicate absorption of dieldrin by earthworms is related to soil type and organic matter (201).

Honey Bees.--- Honey bees are very susceptible to aldrin and dieldrin and there are numerous reports in the literature about honey bee kills related to the use of these pesticides for agricultural purposes. One report indicates that plants treated with dieldrin were toxic to honey bees for up to 9 days (202). There appears to be no information available on the effects if any, of aldrin and dieldrin residues in soil. Specific toxicity information on honey bees (203) has been reported:

oral LD_{50} (µg/bee) is 0.149 for aldrin and 0.150 for dieldrin; LD_{50}/LD_{90} (µg/bee) by topical application to thorax are 0.800/1.175 for aldrin and 0.414/1.202 for dieldrin.

Ostracod.--- Aldrin and dieldrin have 24 hour immobility EC_{50} values of 1.15 and 2.45 ppb, respectively, in Chlamydotheca arcuata (204).

Microorganisms: Toxicities of organochlorine pesticides upon microorganisms involved in ammonification, nitrification, decomposition, and other processes in soil and water have been determined. The studies have concerned survival of bacteria, fungi, and molds following massive disposal of insecticides in soil (205). An application of 200 lb/acre of dieldrin or aldrin is required to depress ammonia or sulfur oxidation in the soil (171). No deleterious effects were observed from dosages of 10 and 20 lb/acre of dieldrin and aldrin on the numbers of bacteria, Streptomyces or molds in soil after 16 months. Toxicity seems to depend on temperature, pH, clay fraction, and organic matter (206).

Annual applications of aldrin and dieldrin for 5 years at rates of 5 lb/acre to two types of field soils exerted no measurable influence on numbers of soil decomposer bacteria and fungi, ten months after the last annual application (171). Aldrin at 10 µg/ml was non-inhibitory to Nitrobacter agilis (207). Doses of 0.1 - 1.0 mg/liter aldrin stimulated growth of saprophytic heterotrophic water microflora (208).

In single and mixed cultures of Streptococcus cremoris and S. lactis, growing in dieldrin at the level of 0.5 and 5 ppm for 5 hr reduced (ca 70%) the rate of lactic acid production, which was restored when milk fat and protein were present (209). After incubation, dieldrin was found tightly bound to the cell surface and was also absorbed into cells. Dieldrin may inhibit acid and alkaline phosphatases as the mechanism of interference with cell metabolism (209).

Degradation studies.--- Both aldrin and dieldrin undergo biotransformations in various fungi, such as Aspergillus niger, A. flavus, Penicillium notatum, P. chrysogenum, and P. vermiculatum. Aldrin is converted to dieldrin and other unidentified compounds. Dieldrin is converted to the similar unidentified compounds (210). Microorganisms which can metabolize organochlorine pesticides, or their analogs, have been diligently researc.. d to discover effective methods of degrading dieldrin and aldrin to non-toxic forms, but only limited success has been attained. Microbes from soil samples have been isolated which are capable of utilizing dieldrin nutritionally, but the degree of degradation and identity of metabolic products were not determined (32). Anderson (211), found indications of dieldrin degradation by the fungus, Mucor alternans. Samples of surface sea water, surface films and open sea water sediments with 0.1 µmole ^{14}C-dieldrin and ^{14}C-aldrin had degradative activity except for open sea water (33). Principal metabolic products were photodieldrin and diol for dieldrin. Aldrin was converted to dieldrin and diol.

Degradation of aldrin by soil microbes has been studied (212). Treatment of twenty isolates previously found capable of utilizing dieldrin or aldrin with 10^{-6} µ aldrin indicated difference among microbes (Table K-10). Metabolites were not identified; therefore, these results show only that the insecticide was microbiologically altered in some way.

TABLE K-10. Comparison of Insecticide Degradation by Soil Microorganisms in Part (212).

Microorganism	Aldrin
Trichoderma viride 12	+
Pseudomonas sp 27	-
Pseudomonas spp 33, 34	-
Trichoderma viride 41	+
Pseudomonas spp 94, 105, 265, 117, 138	+
Micrococcus 204	+
Arthrobacter sp 278	-
Bacillus sp 458	+
Bacillus spp 459, 461	-

Soil flora incubated with 10 or 50 ppm dieldrin labeled in the chlorinated ring with ^{14}C evolved $^{14}CO_2$ (Table K-11) (213). These results that limited attack on the basic ring structure of dieldrin was accomplished by soil microflora.

TABLE K-11. Release of $^{14}CO_2$ from Soil Containing 10 ppm Dieldrin-^{14}C (213)

	$^{14}CO_2$ released/week as % of total Dieldrin-^{14}C							
	Weeks incubation							Cumulative
Soil treatments	1	2	3	4	5	6	7	total
Unsterilized	0.550	0.310	0.217	0.176	0.146	0.327	0.139	1.864
Sterilized	0.200	0.039	0.021	0.014	0.010	0.012	0.007	0.304

Further soil incubation studies were done using 500 ppm ^{14}C-dieldrin in talcum tablets buried in soil with water-soluble metabolites being produced (Table K-12) (213). The 3 most active strains were Nocardia, Corynebacterium, and Micrococcus sp.

TABLE K-12. Microorganisms Producing Water-Soluble Metabolites from Dieldrin -^{14}C, Isolated after 8 Weeks Incubation at 20°C from Talcum Tablets with or without 500 ppm Dieldrin Buried in Moist, Arable Soil (85% WHC) (213).

	Number of Strains with H_2O-Soluble Metabolite					
	Control Tablets			Tablets with 500 ppm Dieldrin		
	Tested	Positive	Maximum*	Tested	Positive	Maximum*
Pseudomonas	11	1	3.3	13	9	21.8
Corynebacterium	15	6	12.0	29	16	15.2
Arthrobacter	21	11	36.5	14	8	15.9
Mycobacterium	14	7	24.3	33	17	23.5
Nocardia	6	5	13.0	9	3	16.0
Mycooccus	4	3	33.4	1	0	0
Micrococcus	0	0	0	1	1	11.5
Bacillus	1	1	3.4	0	0	0
Yeasts	1	1	8.0	2	2	5.5
Total	73	35		104	56	

*Per cent increase in water soluble activity brought about by the most active strain within the group.

Degradation of cyclodiene pesticides by a marine fungus, Falerion xylestrix has been studied (214). F. xylestrix seemed to grow better on culture containing aldrin and dieldrin at 10-50 µg/ml than on glucose alone. Mycelial weight increased linearly at 10-100 µg/ml pesticide, declined at more than 250 µg/ml, and was sparse at 750 and 1000 µg/ml. Maximum growth was achieved in 10 days with 100 µg/ml pesticide. The fungus concentrated approximately 2000x pesticide from growth medium. The organisms showed morphological changes, notably, lack of green pigmentation and the absence of pellets after accumulation of dieldrin or aldrin. F. xylestrix does not use a pesticide nutritionally as no metabolic conversions were observed (214).

Other evidence for pesticide accumulation indicates aldrin-sensitive gram positive bacteria accumulate ^{14}C-aldrin; however aldrin-resistant gram negative bacteria do not (215).

There are few microbes that actually break down dieldrin and aldrin. The process is slow and the metabolic products are close derivatives of the parent compound. Stanford Research Institute studies (216) found no organisms capable of using either pesticide as its sole carbon source, alone, by analog enrichment, or by co-metabolism. However, degradation may proceed too slowly, and reaction products may be beyond detection limits in the experiments. Other studies (213, 214) suggested a breakdown of one or more carbons in the chlorinated ring. Degradation products are mainly photodieldrin from dieldrin and dihydroxyl analogs (e.g. 6,7-dihydroxydihydroaldrin), diol, dieldrin from aldrin, and aldrin from dieldrin (217).

Plants

A review on the sensitivity of ornamental plants to insecticides indicates phytotoxicity of aldrin on azalea, hollyhock, Kalanchoe, narcissus, and Primula (218). Weekly applications of aldrin at 14 pounds active ingredient per acre inhibited root development and size of 2 to 3 week-old tomatoes, cauliflower, and Chinese cabbage plants (219). Normal to 2x normal application decreased percent of germination, retarded growth in beans, soybeans, and cotton (220). Some phytotoxicity was shown in onions dusted with 2.5% (221). Dieldrin (6 pounds dieldrin per acre) injures Dianthus, hollyhock, Kalanchoe, orchids, and Verbina by retardation of growth or leaf injury (218). Beestman, et al. (222) found no phytotoxicity of dieldrin in corn. Normal to 2x normal dieldrin dose is reported to decrease the percent germination and retard growth in beans, soybeans, and cotton (220).

Bioaccumulation: Aldrin and dieldrin have been demonstrated to contaminate many plant species when present in the soil. The contamination of plants from aldrin and dieldrin in soil may come from deposition of aldrin- or deildrin-containing soil particles on aerial plant parts (223), volatilization of the insecticide with penetration of plant cuticle by the vapor (224, 225), and accumulation through roots with translocation to other plant parts (226, 227, 228). The extent of aldrin and dieldrin absorption by plant tissue is dependent upon soil type (22, 222, 228, 229, 230, 231), the species of plant (226, 228, 229, 231, 232, 233, 234) and the concentration of aldrin and dieldrin in the soil.

Plant tissue growing on soil contaminated with aldrin accumulates both aldrin and its epoxide dieldrin. Accumulation has been documented for 5 years after application of aldrin to the soil (230). The more available aldrin is in the soil, the more aldrin and dieldrin is accumulated by plants. For example, potatoes accumulate 10 times more aldrin and 7 times more dieldrin when grown in soil containing 25 pounds per acre aldrin as compared with 5 pounds per acre (229). At least 15 species of vegetables

have been evaluated for aldrin uptake from soil containing the insecticide (229, 230, 234, 235). In addition to these, there are reports on aldrin uptake by alfalfa, sugar beets, peanuts, and peas (229, 230, 234, 236). Carrots and potatoes appear to accumulate the highest concentrations of aldrin and dieldrin from aldrin containing soil (229, 230, 234). In two studies in which 30 pounds aldrin was applied per acre over a three-year period (10 pounds per acre per year) (234, 237), concentrations up to 0.53 ppm aldrin and 0.44 ppm dieldrin were found in the peel of carrots and 0.185 ppm aldrin and 0.212 ppm dieldrin were found in the peel of potatoes. In soil treated with 25 pounds aldrin per acre (229) the residue levels in carrots were 0.94 ppm for aldrin and 0.32 ppm for dieldrin, measured in the growing season aldrin was applied. Accumulation appears to occur mostly in root crops that are in physical contact with soil, since lima beans (229), peas (235), cabbage (230), and broccoli (230) have little accumulation in their edible fractions after application of aldrin at 25 pounds per acre. However, there is some accumulation in aerial plant parts: cucumbers contained 0.17 ppm dieldrin from application of 5 pounds per acre of aldrin (230).

Reports indicate that dieldrin is absorbed by plant roots and translocated to the aerial parts. Investigations on agronomic crops of corn, alfalfa, orchard grass, soybeans, and wheat indicate that all of the crops take up dieldrin from soil (223, 227, 228, 233, 238). With 25 ppm dieldrin in sand, wheat, alfalfa, orchard grass, and corn accumulated approximately 0.45, 0.16, 0.15, and 0.04 ppm, respectively (228) Movement of dieldrin has been demonstrated in wheat to be through the xylem and not phloem (239). Beans from soybean plants grown on soil treated with dieldrin for 5 years had dieldrin residues of 0.76 ppm (238). Highest concentrations of accumulated dieldrin are found in the stem of wheat [25 ppm dieldrin in sand, 2.4 ppm accumulation in stem (228); 2ppm dieldrin in soil, 0.06 ppm to 0.14 ppm in grain, 0.22 to 0.67 ppm in top part of stem, 0.40 to 1.57 ppm in bottom part of stem (227)]. Growth of corn in soil containing 5 ppm dieldrin resulted in up to 7.7 ppm in roots and 2.14 ppm in shoots about 39 days after emergence of the corn plant (222). Concentrations of 24.6 ppm and 0.51 ppm dieldrin were found in roots and shoots of corn respectively, when the plants were grown for 39 days in soil containing 1 ppm dieldrin (222). Dieldrin accumulation has also been studied in carrots (231), radishes (231, 232), turnips (231), onions (231), and sugar beets (232). Highest accumulations occurred in carrots (0.12 ppm from sand containing 0.49 ppm) with lesser accumulation in the other plants (231). Sugar beets contained 0.11 ppm dieldrin accumulated during 19 weeks growth on soil (sandy loam) treated with 4 pounds per acre (232).

Accumulation of dieldrin depends upon the depth of the dieldrin in the soil and characteristics of the soil. Highest residue accumulation came from dieldrin near the surface (1-2 cm) while depths of 16-17 cm and 31-32 cm give considerably less residue in soybeans (240). Highest accumulations in plants appear to occur from growth in sandy soils. In a sandy soil containing 0.49 ppm dieldrin, carrots accumulated 0.12 ppm

(about 1:5); in clay soil containing 0.88 ppm dieldrin, carrots accumulated 0.11 ppm (about 1:9); and in a mucky soil containing 3.87 ppm dieldrin, carrots accumulated 0.02 ppm (about 1:200) (231). Dieldrin uptake by corn has been shown to be inversely correlated with the amount of organic matter in the soil (222).

Beall and Nash (224) demonstrated foliar contamination of soybeans via vapor equal to contamination via root sorption. Soybeans grown in soil containing 20 ppm dieldrin accumulated 0.73 ppm dieldrin through vapor sorption and 0.71 ppm through root absorption (224). Alfalfa grown on soil which had been treated with dieldrin at 5 pounds per acre 3 years previous had residues of 0.016 to 0.039 ppm probably due to splashing of dieldrin-contaminated soil onto foliage (223).

Total dieldrin residue found in plant tissue may result from dieldrin uptake and conversion of absorbed aldrin to dieldrin in plant tissue.

Degradation: Within various plant tissue examined, the major degradation product (70-80%) of aldrin appears to be dieldrin (22, 241). Some reports indicate that other hydrophilic metabolites are also formed (22, 24, 41). Dieldrin is not readily degraded in plant tissue, although some dieldrin-related alcohols and ketones are found in plant tissue treated with dieldrin (241).

Food Chain

Bioaccumulation of both aldrin and dieldrin can occur through uptake by plants (242). Concentrations of aldrin and dieldrin in carrots (after growth in soil treated with 25 pounds aldrin per acre) contained over 9 times the accepted residue levels (0.1 ppm) for most vegetable crops (229). Residue levels of aldrin in tissue were also greater than 0.1 ppm in carrots treated with only 5 pounds per acre (229). Growth of food and feed plants on soil containing aldrin could lead to consumption of aldrin and dieldrin by man and animals. The higher the concentration of available aldrin and dieldrin in the soil, the higher the risk of aldrin and dieldrin accumulation in plant tissue. Aldrin is converted to dieldrin as it moves through the food chain. Worms were found to have 2 to 10 times higher concentrations of dieldrin than were present in the ambient soils (138).

Biomagnification readily occurs in fish and snails (243). Laboratory experiments indicate that fish can build up dieldrin to ppm levels from water containing parts per trillion (244). Sailfin mollies exposed to 7.5 ppb had dieldrin concentrations in the gut, liver and muscle ranging from 2.0 to 8.0 ppm after 144 hours. The mortality rate accelerated after twenty-four hours when all sampled tissues contained between 5 and 11 ppm of dieldrin (245).

Ultimately dieldrin enters the food chain from both soil and water. In a model ecosystem containing land plants, algae, snails and fish, the movement of aldrin and dieldrin have been followed. Aldrin-^{14}C and dieldrin-^{14}C were applied at levels equivalent to 1 lb/acre. After 33

days, analysis of the organisms were made and the distribution of aldrin and dieldrin determined. Data are presented in Table K-13 (243).

TABLE K-13. Transport of Aldrin and Dieldrin Through a Food Chain in a Model Ecosystem (243).

		Aldrin Equivalents (ppm)				
		H_2O	Algae	Snail	Mosquito	Fish
	Total ^{14}C	0.0117	19.70	57.20	1.13	29.21
Aldrin Applied	Aldrin	0.00005	1.95	2.23	--	0.157
	Dieldrin	0.0047	16.88	52.40	1.1	28.0
	Unknown I	--	0.57	2.05	--	0.612
	Unknown II	0.00039	0.015	--	--	--
	Total ^{14}C	0.0039	0.73	90.09	--	3.96
Dieldrin Applied	Dieldrin	0.0014	0.64	86.32	--	3.78
	9-OH-Dieldrin	0.00009	0.03	0.51	--	0.07
	9-C=O Dieldrin	--	--	0.42	--	0.023
	Sum of 5 Unknowns	0.00149	0.0481	2.22	--	0.0518

Biomagnification of both dieldrin and photodieldrin occurs in algae (Ankistrodesmus amallides) (246).

Movement of dieldrin into foods has also been found (31). Some of the levels are shown in Table K-14.

EXISTING STANDARDS

Existing standards for both aldrin and dieldrin are: threshold limit value (TLV) of 0.25 mg/m^3 (247); drinking water standard of 0.001 ppm (124); and acceptable daily intake (ADI) of 0.0001 mg/kg/day (248).

TABLE K-14. Concentration of Dieldrin in Foods (31).

Nature of Sample	Number of Specimens	Lower Limit of detection, ppm	Dieldrin content ppm
English mutton fat	2	0.001	0.07
Australian mutton fat	1	0.0001	0.01
Argentine corned beef fat	2	0.002	0.16 & 0.015
Crude & refined edible oils & fats	8	0.004-0.05	0.05
Whole cooked meals	2	0.001	0.02
Butter	2	0.001	0.04
Cooked meats	2	0.001	0.008
Milk	1	0.001	0.006
Shag cormorant eggs	62 (pooled)	0.0001	2.1
Potatoes	2	0.005	0.04
Forage beet foliage	1	0.01	0.09
Forage beet	1	0.008	0.005

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APPENDIX L

CHLORDANE

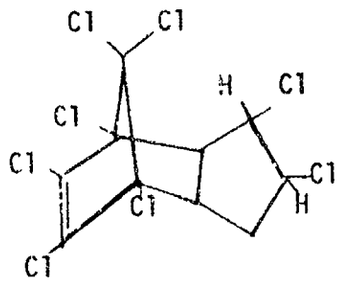
ALTERNATIVE NAMES

α-(cis)-4,7-Methano-1H-indene. 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-, (1α,2α,3aα,4β, 7β, 7aα)-;β-(trans)-4,7-methano-1-H-indene, 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-, (1α, 2β, 3aα, 4β, 7β, 7aα)-(Chem. Abstr. after 1971); 4,7-methanoindan-1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-(Chem. Abstr. 1962-1971); chlordan (Chem. Abstr. before 1962); chlordane; gamma-chlordan; CD 68; chlorodane; ENT 9,932; M 140; M 410; octachlor, octachlorodihydrodicyclopentadiene-1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene; octachloro-4,7-methanotetrahydroindane; Octaklor; Ortho Klor; Synklor; Tatchlor 4; Toxichlor; Velsicol 1068.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Reg. No. 57-74-9
5147-74-9 (alpha),
5103-74-2 (beta)
Toxic Substances List: PB9800
Wiswesser Line Notation: L C555 A IUTJ AG AG BG DG EG HG IG JG
Molecular formula: $C_{10}H_6Cl_8$

Structural formula:



Technical chlordane is a mixture of isomers and related compounds. The approximate composition of chlordane is shown in the table below.

TABLE L-1. Approximate Composition of Technical Chlordane[a] (1).

Fraction	Percentage Present
Diels-Alder adduct of cyclopentadiene and pentachlorocyclopentadiene ($C_{10}H_7Cl_5$)	2 ± 1
Chlordene ($C_{10}H_6Cl_6$); isomer 1	1 ± 1
Chlordene isomers 2, 3 and 4 together	7.5 ± 2 13 ± 2
Heptachlor ($C_{10}H_5Cl_7$)	10 ± 3
cis-Chlordane ($C_{10}H_6Cl_8$) (β)	19 ± 3
trans-Chlordane ($C_{10}H_6Cl_8$) (α)[b]	24 ± 2
Nonachlor ($C_{10}H_5Cl_9$)	7 ± 3
Hexachlorocyclopentadiene (C_5Cl_6)	>1
Octachlorocyclopentene (C_5Cl_8)	1 ± 1
$C_{10}H_{7-8}Cl_{6-7}$	8.5 ± 2
Consiituents with shorter GC retention time than C_5Cl_8 (includes hexachlorocyclopentadiene)	2 ± 2
Constituents with longer GC retention times than nonachlor	4 ± 3

[a]Adapted from data of Velsicol Chemical Corporation.

[b]This is referred to as γ-chlordane by Velsicol.

Chlordane manufacture involves a Diels-Alder addition of hexachlorocyclopentadiene and cyclopentadiene. The adduct (chlordene) is dissolved in CCl_4 and treated with chlorine gas, with the resultant addition of two atoms of chlorine to the double bond to form chlordane (2, 3). Technical chlordane is a viscous, amber-colored liquid (2). There is only one manufacturer of chlordane in the U.S., the Velsicol Corporation of Chicago. Julius Hyman Co., of Denver, Colorado made chlordane until 1950 (2).

TABLE L-2. Chemical and Physical Properties.

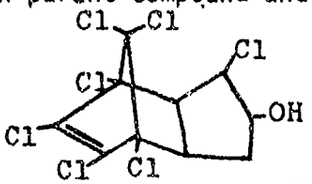
Boiling point:	175°C at 2 mm Hg (2) 118°C at 0.06 mm Hg (4)		
Solubility:	Insoluble in water, soluble in aliphatic, aromatic and chlorinated hydrocarbons (2)		
Specific gravity:	1.59-1.63 at 25°C (4)		
Vapor pressure:	1×10^{-5} mm Hg at 25°C (4)		
Behavior to chemicals:	Acids	Stable	(4)
	Mild alkali	Stable	(4)
	Strong alkali	Dehydrohalogenates	(4, 5)
	Zn/acetic acid	Partially dehalogenates	(6)
Carbon adsorption:	50 ppm of chlordane in water treated with 10 ppm activated carbon resulted in 99% removal of chlordane (5)		

Photolysis: Benson et al. (7) have described the preparation and characterization of photolysis products of chlordane isomers and technical chlordane by ultraviolet irradiation, including that of sunlight. Irradiation of cis-chlordane by UV-light yields a bridged photo isomer, whereas, the trans isomer does not undergo change (1). Technical chlordane showed a high rate of decomposition in sunlight; this was based upon bioassay measurement (8).

Metabolism: Reports of the metabolic transformations of chordane suffer a certain degree of confusion owing to the use of three nomenclature systems for the two principal isomers of chlordane. The stereochemically unambiguous system uses cis- and trans- chlordane as the respective names for what were called α- and γ-chlordane by the original manufacturers, and β- and α-chlordane by various investigators, beginning about 1952. It is sometimes difficult to tell in earlier work just what material was involved. An FAO/WHO monograph (9) summarizes the situation and shows the structures of the mammalian metabolites known at that date, 1973.

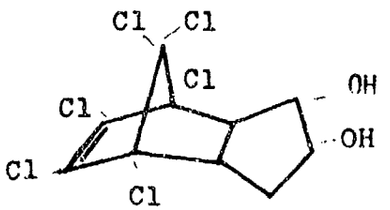
The review of Brooks in 1969 (10) goes over earlier work (1950's), and contains references to this information.

In rats and rabbits, trans-chlordane is converted to hydrophilic substances that are excreted in urine and feces. One compound, trans-1-hydroxy-2-chlorodihydrochlordene, shown below, was found to some extent in the abdominal fat of the rabbit, following repeated oral doses, but not in subcutaneous fat. Chlordane itself was found in both types of fat (Poonawalla and Korte, 1971 (11)). A number of rabbit tissues contained varying proportions of both parent compound and metabolite.



trans-1-Hydroxy-2-chlorodihydrochlordene

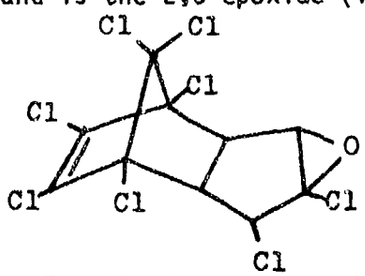
A second metabolite, the 1,2-dihydroxydihydrochlordene, shown below, is excreted in lesser amounts in rabbit urine (10).



1,2-Dihydroxydihydrochlordene

These hydrophilic metabolites perhaps explain why chlordane has not been detected in many samples of human fat during various surveys for pesticide residues in man.

A third metabolite, oxychlordane, shown below, is derived from either cis- or trans-chlordane and is the 2,3-epoxide (12).



Oxychlordane

This material, which in earlier papers may have been mistakenly identified as heptachlor epoxide, has been isolated from fat of rats, dogs, cattle, and pigs (12, 13, 14, 15), and from the milk of cows fed alfalfa contaminated with chlordane (15, 16). Polen (13) reported fat levels of 8-22 ppm oxychlordane in rats given 5-45 ppm of α-chlordane (cis-isomer) for one year; and a concentration of oxychlordane equal to the concentration of γ-chlordane (trans-isomer) fed during the year, i.e., there was a storage-to-diet ratio of one for the trans-isomer, but generally less than one for the cis-isomer. In dogs fed technical chlordane for two years, the oxychlordane fat storage-chlordane feed ratio was also one (13).

Likewise, Street and Blau, 1972 (17) reported that the rat stored more oxychlordane after being fed trans-chlordane than after being given the cis-compound; and males stored less oxychlordane from either isomer than did females. But liver homogenates incubated with chlordane isomers produced oxychlordane in a manner to suggest that dichlorochlordene was an intermediate.

The very recent report of Barnett and Dorough, 1974 (14) indicates oxychlordane to be the most persistent residue in the tissues after chlordane is removed from the diet. Pure cis-trans isomers and a high-purity (98+%) product, containing a 3:1 mixture of cis- and trans-chlordane (all ^{14}C-labeled) were used in the study. Trans-chlordane gave higher tissue residues than did the cis-isomer.

Biros and Enos, 1973 (15) reported 0.14 ppm of oxychlordane in 78% of 27 human fat samples examined, and suggested the inclusion of this metabolite in the general monitoring program for pesticide exposure in man. The range of oxychlordane was found to be 0.003 to 0.40 ppm in the fat samples.

Polen (13) reported finding no evidence of oxychlordane in plants or soil treated with technical chlordane.

Microsomal enzyme induction by organochlorine insecticides is now well-established. The original observation of this phenomenon for these insecticides was that of Hart, et al., (18) and there has been much work in this area since. Hart and Fouts (19) have indicated that chlordane is non-specific, resembling phenobarbital in this respect. A brief but useful review on this subject is that of Conney, et al. (20).

ANALYTICAL METHODS

Prior to gas chromatography, measurement of chlordane on crops was done using the Davidow Reagent, a colorimetric test with sensitivity of 2.5 to 5 μg of technical chlordane, or 0.025-0.05 ppm based on a 100-g sample (1). Anotner colorimetric method is the Polen-Silverman method (21).

In addition to these, a bioassay method has been described (22). Inhibition of hexokinase by chlordane has been used to measure chlordane, with the lowest detectable concentration being 2.7×10^{-6} M; aldrin and DDT interfere with this method (23). Several thin-layer chromatographic (14, 24, 25, 26, 27, 28) methods sensitive to about 0.02-0.5 μg, and one paper chromatographic procedure (29) have been described.

The best method for analysis is gas chromatography using electron capture (EC) or microcoulometric (MC) detection of components. Coupled with mass spectrometry (MS), use of gas chromatography permits absolute identification. Mass spectra of chlordane have been published (30, 31). Table L-3 presents the gas chromatographic analyses of chlordane residues in various types of materials. Being a mixture of substances, chlordane residues exhibit two prominent peaks ("signature peaks") in the gas chromatogram (1).

TABLE L-3. Chromatographic Analysis for Chlordane.

Type of Sample	Clean-Up	Detector	Limits of Detection	Reference
Potatoes	None	EC	0.01 ppm	32
Soybeans	Florisil	EC	Not Set	33
Soil				
Fish	None	EC	0.01 ppm	34
Soil	None	EC	Not Set	35
Soil	Florisil	EC	0.01 ppm	36
Sugarbeets, Soil	None	EC	0.01 ppm	37
None	None	TC	Not Set	38
Broccoli, Lettuce	None	MC	Not Set	39
Vegetables	Channel-chromatography	EC	0.03 ppm	40
Wastewater	TLC	EC/MC	0.0015-0.022 ppm	41
Blood	Extraction	MC	15 ng	42

Since artifacts and some plant extracts have the same or similar retention times as those of certain pesticides, the gas chromatographic identification should be carried out with a confirmatory test. Mass spectrometry is the most decisive method for confirmation (43, 44). In addition, there is the possibility of chemical derivatization of chlordane coupled with gas chromatography as a check on its identity. For this purpose, potassium t-butoxide in t-butanol is used to convert cis-and trans-chlordane to 3-chloro and 2-chlorochlordene, respectively (45, 46).

MAMMALIAN TOXICOLOGY

Human Exposures

Accidental ingestion of or skin contamination with technical chlordane has caused death or severe poisoning, with convulsions, deranged vision, vomiting, hyperexcitability, etc. A dose estimated to have been about

100 mg/kg proved fatal. In an 18-year old woman, 30 mg/kg (estimated) produced convulsions and lesser symptoms, with complete recovery. It was thought that some two-thirds of the dose was vomited within five hours after ingestion (Dadey and Kammer, 1953 (47)). Curley and Garrettson, 1969 (48), reported on a two-year old boy who recovered after serious poisoning. Serum chlordane reached 2.7 mg/l shortly after the last convulsion, dropped to 0.2 mg/l by day 9, and to 0.02 mg/l by day 95.

Chronic toxicity: Workers engaged in the manufacture or formulation of chlordane for up to 15 years have shown no evidence of harmful effects (Princi and Spurbeck, 1951 (49); Alvarez and Hyman, 1953 (50)). Only three cases of chlordane intoxication were reported out of 1105 persons surveyed who had been engaged in pest control work for 1-30 years (FAO-WHO report, 1973 (9)). No chlordane was found in over 200 human autopsy fat samples (FAO/WHO report, 1973 (9)), although other insecticides were regularly detected.

Experimental Animals

Acute toxicity: Acute oral LD_{50}'s for technical chlordane have been collected in various handbooks and review articles (e.g., Handbook of Toxicology, Volume 3, 1959 (51), Gaines, 1969 (52), Welch, 1948 (53)). They range (in mg/kg for various species) from 100-300 for the rabbit to 200-750 for the rat, to 130 for the goat and 500-1000 for sheep, depending on the solvent used, strain of animal, etc. It must be borne in mind that earlier toxicity data are suspect because of the variability in composition of technical chlordane before about 1953 (9).

In the newborn rat, chlordane is less toxic acutely than in the adult, the MLD's (presumably oral, though not so stated) being, respectively, 1121 and 344 mg/kg (Harbison, 1973 (54)). Pretreatment with phenobarbital enhances chlordane toxicity in the newborn, the MLD decreasing to 539 mg/kg.

Dermally, the material is readily absorbed; acute LD_{50}'s by this route have been quoted as 690-840 mg/kg in the rat (Gaines, 1969 (52)). By contrast to these single-dose dermal figures, 50 mg/kg applied daily for four days to the rat and 20-40 mg/kg as repeated daily exposures in the rabbit are quoted as LD_{50} values. In other animals, successive sprayings or dips in 1.5-2% emulsions of chlordane were fatal to goats, sheep, and cattle (51, 55).

A high toxicity had at one time been reported for mice and birds exposed to chlordane vapors, but later work (Ingle, 1953 (56)) showed this to be due in all likelihood to the presence of hexachlorocyclopentadiene in the product tested earlier. Subsequent investigations have failed to show inhalation toxicity from the technical material.

Subacute oral studies in which 6-25 mg/kg of chlordane were given to rats daily for about two weeks did not produce convulsions, but doses of 50 mg/kg caused toxic signs and the animals died (Ambrose et al., 1953 (57)). In a 9-month feeding study, 2.5-25 ppm technical chlordane produced

hepatic cell hypertrophy and other histological changes even at the low dose (Ortega et al., 1957; quoted in FAO/WHO report, 1973 (9)).

Chronic toxicity: When dogs were given 80-3200 ppm chlordane in the diet for the survival time of the animal, all dogs at 800, 1600, and 3200 ppm died in 8 weeks; at 400 ppm the animals survived 26 weeks; and at 80-200 ppm they went 53-90 weeks. Body weight gain was decreased at all levels. Fatty degeneration of the liver was seen in the dogs examined histopathologically (200 ppm) (Lehman, 1965 (58)).

Chronic feeding studies in the rat have been carried out for as long as two years. Ingle (59) reported increased mortality and reduced body weight gain at 150 and 300 ppm, but not at 5, 10, and 30 ppm. There were liver and kidney enlargement at the two highest feeding levels, with liver cell hypertrophy, centrilobular necrosis, and change in kidneys, adrenals, lungs, and duodenum. These changes were marked only at 150 ppm and above. Young rats nursed by mothers ingesting 150-300 ppm chlordane showed signs of toxicity, including death.

A two-year rat study summarized by Lehman in 1965 (58) showed increased liver-body weight ratios as low as 25 ppm in the males, and specific though minimal hepatic cell changes at 2.5 ppm. A second long-term rat study by Ingle (9) failed to show liver cell changes at 2.5-25 ppm levels, and only slight changes at 50 ppm. At 75-300 ppm the changes were those described in Ingle's 1952 paper (59).

A further publication by Ingle (9) reported feeding experiments in which 5-35 ppm of cis-chlordane, 15-75 ppm of trans-chlordane or 5-50 ppm of a 1:1 mixture of the two isomers were fed to rats for 78 weeks. There was increased mortality in both sexes at 35 ppm of cis-chlordane and 75 ppm of trans-chlordane, as well as at 50 ppm of the mixture. Growth was decreased only at the highest level of each material. There were no hematological changes and no gross pathology--including tumors. No histopathological changes were seen below 25 ppm in any case, but slight-to-moderate hepatic changes at 25 ppm (and above) for the cis-isomer, at 35-75 ppm of the trans-isomer, and at 50 ppm for the mixture.

In the dog, 0.3, 3, 15, and 30 ppm of chlordane were fed for two years (9). Clinical liver function tests gave abnormal results at the 15 and 30 ppm levels, and increased relative liver weights were seen at termination in these two groups. Periodic liver biopsies showed hepatocellular changes at 6 months, but not before. No effects on body weight gain, behavior, survival or hemograms were found at any level.

A three-generation rat reproduction study was conducted by Ingle (9). Levels were 0.3-60 ppm. Levels up to and including 30 ppm had no effect in any generation on fertility, litter size, pup body weights, mortality or growth through weaning. There were no histopathological changes in the weanlings at autopsy. At 60 ppm, however, about 11% mortality was

seen in the second F_3-generation litters during the nursing period. The pups showed gross and histologic changes appropriate to chlordane toxicity. Other F_3 litters, from 60-ppm dams that had been placed on control diet for 30 days prior to mating, were comparable to control litters in all respects. No evidence of teratogenicity was seen.

Changes in conditioned reflexes in cats given 25 mg/kg were observed; also 10-15 mg had effects, but recovery was faster. In dust form, 0.01 mg/liter resulted in changes in white cells; 0.002 mg/liter resulted in changes in the conditioned reflexes (60).

Carcinogenicity: No carcinogenic studies per se and no mutagenic experiments seem to have been published. None of the chronic animal experiments have shown any evidence of carcinogenicity. However, a memorandum from U. Saffioti (61) to the Chairman of the DHEW Committee to Coordinated Toxicology and Related Programs indicates soon-to-be completed studies in mice have demonstrated carcinogenicity for chlordane (and heptachlor, which is a component of technical chlordane).

Metabolites and photoproducts: The metabolism of chlordane in experimental animals has already been described in the section on "PHYSICAL AND CHEMICAL PROPERTIES". The acute oral toxicity of the 1-hydroxy-2-chloro metabolite is said to be lower than that of chlordane: LD_{50} in mice about 1800 mg/kg.

The acute oral LD_{50} of oxychlordane in the rat is quoted (from unpublished work) in the FAO/WHO monograph, 1973 p. 36 (9) as 19 mg/kg.

No chronic toxicity studies appear to have been done with the metabolites. The FAO/WHO monograph, 1973 (9), suggested that the low acute toxicity of the hydroxy compounds and the absence of oxychlordane in extracts from plants or soil made such studies unnecessary.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Leaching and Persistence in Soil: The leaching index for chlordane indicates less than 10 cm movement through soil with a rainfall of 150 cm per year (62). The rate of loss probably depends on the type of soil, the climate, the depth, and other factors. Although one author gives a half-life of 2-4 years for the disappearance of chlordane in soil (63), this seems too low in view of other data. Another author reports 4 to 5 years (37). Thirteen years after application of chlordane to 38 cm of Congaree sandy loam outdoor plots, 64% of the applied chlordane was still present and 90% present found in the top 25 cm of soil (64). The disappearance of a number of pesticides in Congaree sandy loam plots was followed over a 14-year period. After 14 years, 40% of the initial chlordane concentration was still present (65). In another study with Congaree sandy loam, 7% of the original chlordane was present after 16 years (66). Sixteen years after 224 kg/ha of chlordane were applied to a soil,

3.8 ppm and 3.15 ppm of cis- and trans-chlordane, respectively, 0.20 ppm of heptachlor, and 0.33 ppm of heptachlor epoxide were found in the soil (66). After the application of technical chlordane to field soil, successive analyses for residues showed rapid disappearance of all minor components, leaving trans- and cis-chlordane (1). Twenty-one years after chlordane application to the perimeter of a building for termite protection, chlordane was still present at 15% of the original level. The greatest concentration was at the point of application. Table L-4 shows the concentration profile of soil and the decrease in concentration with distance from the building.

TABLE L-4. Horizontal and Vertical Distribution of Gamma Chlordane Residues in Treated Soil Around a Building (36).

Core Segment	Segment Depth (in)	Residues (ppm) Distance from Building (ft) 0.5	1	2	4	10
A	0 - 5	0.83	0.39	0.42	0.34	0.07
B	6 - 10	0.04	0.01	0.01	<0.01	<0.01
C	11 - 15	<0.01	<0.01	<0.01	<0.01	<0.01
D	16 - 20	0.01	<0.01	0.03	<0.01	<0.01

This shows that chlordane is quite persistent and exhibits little vertical or horizontal movement in soil under natural conditions.

Vaporization: The vaporization of chlordane from soil is one path of loss. The vaporization index for chlordane indicates a loss of 0.2-3 kg/ha/yr (62). During a laboratory study of volatilization, the ratio of trans- to cis-isomers remained constant (67).

Water: The concentration of chlordane in U.S. surface water is estimated to average 0.169 ppb (68).

Animals

Mammals: Data for mammals are covered under *"Experimental Animals."*

Birds: Reactions of birds to chlordane are variable, depending on the nature of exposure. To turkey poults, 2% spray is toxic (69). Surface applications of 5% on walls of pens, which resulted in vaporization, did not harm chicks (70) or pigeons (71). The effects of chlordane in combination with other insecticides tend to be additive rather than synergistic in Japanese quail and pheasants (72).

Fish: Some fish are very sensitive to chlordane, e.g., bass fingerlings are affected by 0.2 ppm (73), trout by 0.25 µg/l (74). The LC_{50} values for chlordane to fish are given in Table L-5.

TABLE L-5. LC_{50} of Chlordane in Fish

Species	LC_{50} (µg/l) 96 hrs	References
Salmo gairdneri (rainbow trout)[a]	1-30	74, 75
Micropterus salmoides (black bass)[b]	200	73, 75
Lepomis microchirus (bluegill)[c]	200	75
Lepomis microchirus (bluegill)	22	76
Lepomis microchirus (bluegill)	77-85	77
Ictalurus punctatus (channel catfish)	500	75
Pimephalus promelas (fathead minnow)	52	75
Cyprinus carpio (carp)[d]	1,160	78
Crassius auratus (goldfish)	50-82	76
Crassius auratus (goldfish)[e]		79

a 24 hr study
b 30 hr study
c 87 hr study
d 48 hr study
e Positive response to 2,000 µg/l. No reaction was observed to 100 ppb in foods.

Freshwater Invertebrates: See Table L-6.

Table L-6. TL_{50} Values of Chlordane in Aquatic Crustaceans (80).

Species	TL_{50} µg/l[a] 24 hrs	96 hrs
Gammarus fasciatus	100 (60-190)	40 (21-60)
Palaemonetes kadiakensis	120 (90-160)	10 (7-13)

[a] 95% confidence limits in parenthesis.

Earthworms: Chlordane treatment with 12-24 lb/acre (emulsions) or 16-32 lb/acre (granules) is toxic to earthworms (81). Worms may contain residues 4 to 9 times greater than those in surrounding soil (82, 83).

Honey Bees: Chlordane is toxic to honey bees as follows: Oral LD_{50} (µg/bee) is 0.900; tarsal LD_{50} (µg/bee) is 0.514; and LD_{50} for bees exposed to chlordane-impregnated filter paper (µg/100 sq cm) is 16.952.

Microorganisms: In field applications of chlordane, 500 lb/ acre of the pesticide were required to reduce numbers of fungi and nitrifying organisms in the soil (83). Martin (83), citing his previous study, indicated that 10 lb/acre chlordane, applied in 5 annual applications, had no measurable effect on numbers or function of the soil population. Martin's review further indicated that 200 lb/acre chlordane were needed to depress ammonia and sulfur oxidation in soil.

Winely and San Clemente (85) found that 10 µg/ml chlordane prevented growth of Nitrobacter agilis in liquid suspension. Chlordane was found to be more toxic to nitrite oxidase in cell-free extracts than in whole cells, where only partial inhibition of oxidation occurred. Chlordane did not inhibit cell-free nitrate reductase, but did cause some repression of cytochrome oxidase activity.

In a review by Bohonos and Francis (86) there is evidence that technical chlordane inhibits the growth of gram positive bacteria and that NADH formation is inhibited in membrane preparations of Bacillus subtilis. Chlordane specifically interfered with oxidative metabolism of Saccharomyces cerevisiae.

Degradation: Degradation of chlordane by microorganisms is almost unknown. Iyengar and Rae (87) found that Aspergillus niger can utilize chlordane in culture. Table L-7 illustrates their work.

Table L-7 Utilization of Chlordane by A. niger in 48 hr.

Concentration of Pesticide in Medium		Utilization
Initial	Final	%
12.5	Not detectable	100
25.1	0.5	98
37.5	3.0	92
50.0	6.25	87.5

Chlordane above 50 µg/ml inhibited growth. Results also indicated that chlordane could not serve as the sole carbon source for A. niger. Once accustomed to growth in chlordane, A. niger could also utilize other structurally similar cyclodienes. Metabolites were not identified (87).

Plants

Phytotoxicity: Chlordane in the soil can be phytotoxic. There are differences in sensitivity to chlordane among plant species and the phytotoxicity of chlordane is also dependent upon the concentration of chlordane in the soil (more chlordane, more toxicity) (88, 89). Chlordane has been used as a selective herbicide for crabgrass control in stands of turfgrass (88, 89, 90). Applications of 65 pounds per acre of chlordane reduced a stand of Kentucky bluegrass 95 percent, while application of 260 pounds per acre of chlordane reduced growth of annual bluegrass 59 percent (89). Application of chlordane at 130.5 pounds per acre for 3 years had no residual effects on vigor of radishes grown in the soil (90). Hagley (91) evaluated the effect of chlordane on growth of foliage and roots of some vegetable crops. With treatment of soil at 1.4 pounds per acre, chlordane reduced the foliage growth of Chinese cabbage, but had no effect on cauliflower or tomato growth at 14 pounds per acre. Foster (92) tested the phytotoxicity of chlordane at up to 400 pounds per acre on melons, corn, cucurbits, tomatoes, beans, beets, and cabbage. The growth of honeydew melons was depressed by 25 pounds of chlordane per acre and growth of cucurbits, tomatoes, and beets was depressed by 400 pounds per acre. Beans, cabbage and corn appeared tolerant of large amounts of chlordane. However, lima beans are injured by aerial spray of 5 pounds/100 gal (93). Sudan grass and tomato growth was retarded by applications of 120 lb/acre (94).

Bioaccumulation: Chlordane residues are taken up from soil into plant tissue. Onsager et al. (37) found that with sugar beets the chlordane residue averaged 9.6% of the concentration in the soil. With potatoes growing on soil containing 10 pounds chlordane per acre, residues of 0.08 ppm amounted to about 3% of the concentration of chlordane in soil (22). Table L-8 presents the analysis of whole potatoes grown in soils treated with two levels of chlordane (32).

TABLE L-8. Chlordane Residues in Potatoes Grown in Chlordane Treated Soils (32).

Soil Dosage	Residues (ppb)
4 lb/acre	75
6 lb/acre	76 & 100

In another study, the translocation of chlordane into vegetables was found. Table L-9 presents the data (95).

Table L-9. Residues of Chlordane in Crops Grown in Soils Treated with Technical Chlordane at 6 lb/acre (95).

Crop	Residues (ppm) Residue Station 1967	Residue Station 1968	Farm 1968
Beets	0.01 ± .001	--	0.03 ± .004
Carrots	0.07 ± .005	0.05 ± .01	0.26 ± .04
Parsnips	--	0.12 ± .02	0.24 ± .03
Potatoes			
Peel	0.15 ± .007	0.11 ± .03	0.26 ± .04
Pulp	N.D.	N.D.	N.D.
Whole Tuber	0.03	0.02	0.04
Rutabagas			
Peel	0.02 ± .002 0.10 ± .01	0.05 ± .007	0.07 ± .007
Pulp	N.D	N.D.	N.D.
Whole root	0.01	0.01	0.01

N.D. = not detectable (<0.005 ppm)

Treatment of soil with 10 pounds per acre gave residues of 1.51 ppm, 0.50 ppm and 0.43 ppm in carrots, lettuce and rutabagas, respectively (96). Several other vegetables showed lesser residue levels (<0.2 ppm) (96). Application of chlordane to plots (Congaree sandy loam) at 56, 112, and 224 kilograms per acre resulted in residues of 9% of the original material 16 years later (3.2 ppm cis- and 3.8 ppm trans-chlordane), but no accumulation of chlordane was detectable in seeds of soybeans grown on these plots (66). Soybeans grown in various areas of South Carolina, however, contained between 0.001 and 0.212 ppm of chlordane (33). In his review on plant uptake of pesticides, Nash (97) suggests that chlordane is absorbed by plant roots but it is very improbable that there is any translocation of chlordane from roots to aerial parts of the plant. However, translocation of chlordane (unspecified amount) into alfalfa foliage has been reported (98).

Degradation: Chlordane is apparently metabolized in plant tissue with the major residue being cis-, trans-, photo-cis-, and oxychlordane. Photo-cis, and oxychlordane accounted for 16 and 17%, respectively, of chlordane residues in alfalfa grown on soil previously treated with 10 lb/acre chlordane (98).

EXISTING STANDARDS

Existing standards for chlordane are: Threshold limit value (TLV) of 0.5 mg/m^3 (99); drinking water standard of 0.003 ppm (100); and acceptable daily intake (ADI) of 0.001 mg/kg/day (101).

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APPENDIX M

ENDRIN

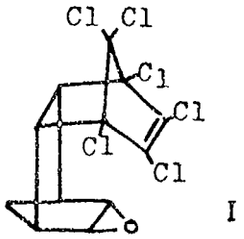
ALTERNATIVE NAMES

2,7:3,6-Dimethanonapth[2,3-b]oxirene, 3,4,5,6,9,9-hexachloro-1a,2,2a, 3,6,6a,7,7a-octahydro, endo endo (Chem. Abstr. after 1971); 1,4:5,8-dimethanonapthalene-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7, 7,8,8a-octahydro (Chem. Abstr. after 1962); endrin (Chem. Abstr. before 1962); Experimental Insecticide 269; hexachloroepoxy-octahydro-endo-endo-dimethanonaphthalene.

PHYSICAL AND CHEMICAL PROPERTIES

CAS Reg. No. 72-20-8
Toxic Substances List: IO 15750
Wiswesser Line Notation: T E3 D5 C555 A D-FO KUTJ AG AG BG JG KGO LG
Molecular formula: $C_{12}H_8Cl_6O$

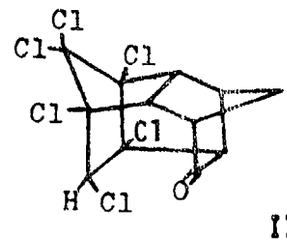
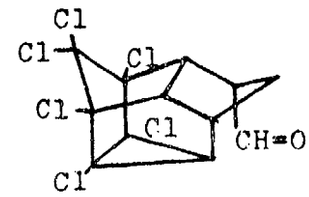
Structural formula:



I

Endrin is made by the epoxidation of isodrin with peracetic or perbenzoic acid. Isodrin is made by the slow reaction of cyclopentadiene with the condensation product of vinyl chloride and hexachlorocyclopentadiene (1). Endrin is the geometrical isomer of dieldrin. Selected chemical and physical properties are presented in Table M-1.

TABLE M-1: Physical and Chemical Properties of Endrin

Property		Reference
Melting point pure, °C	235°, with decomposition	(2)
Vapor pressure	2×10^{-7} torr at 25°C	(2)
Specific Gravity	1.645 at 25°C	(2)
Heat Stability	Rearranges rapidly above 200°C to a half-cage ketone and an aldehyde	(3)
	 II and  III	
Chemical Stability	Alkali - Stable	(2, 4)
	Acids - Rearrangement to half-cage ketone	(5)
	$KMnO_4$(50 ppm) Inert	(6)
	Cl_2(61 ppm) Inert	(6)
Solubility in Water	0.23 ppm, 25°C 0.38 ppm, 35°C 0.51 ppm, 45°C	(7) (7) (7)
Solubility in Organic olvents	Moderately soluble in acetone and benzene. Sparingly soluble in alcohols, paraffins and xylene	(2)
Activated Carbon	Endrin in water in the 0.5-10 ppb range could be reduced to 0.25 ppb by 30-60 ppm powdered activated carbon	(4)

Endrin in the solid state is readily photolyzed by sunlight; the major product is the ketone (II), in 37% yield; the aldehyde (III) is formed in 9% yield (8). Complete conversion of endrin was possible in 17 ± 2 days in an intense summer sun (8). The rate data in this study are shown in Table M-2.

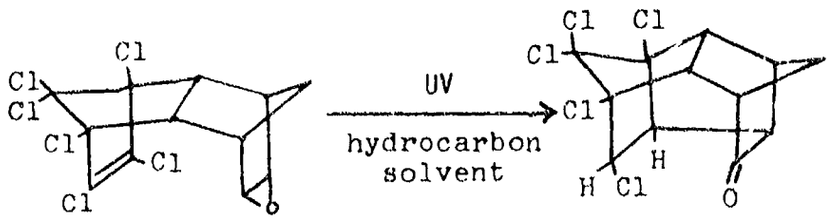
TABLE M-2: Endrin Photoproduct Formed vs. Sun's Intensity (8)

Days Exposed	Month	% Isomeric Ketone II Formed
5	October	14
5	June	46
12	October	30
12	June	65

The same products result from artificial UV irradiation of endrin (9). Rotenone catalyzes the photochemical conversion (10).

Even if only deposited on air-dried clays, endrin may isomerize to the aldehyde (III) and the ketone (II) in 24 hours (11).

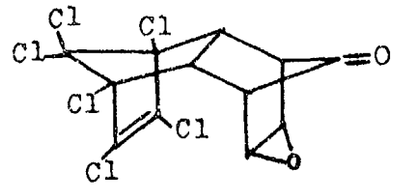
In addition to the above photoproducts, another has been found on irradiation of endrin in a hydrocarbon solvent. This photoproduct (shown below) may be separated from endrin by gas chromatography using an 11% OV-11/QF-1 column. It has been found in field soils (12). Unlike the other photoproducts (8), this one is a result of photodechlorination.



IV

Endrin is degraded by the same microorganisms that degrade dieldrin. Ketoendrin (II) is a metabolite (13). Of 150 microbial isolates from soils, 25 actively degraded endrin (14). At least 7 metabolites, 3 major ones, were isolated from a culture of *Pseudomonas;* one of the metabolites was ketoendrin (II) (14). The action of anaerobic sludge was effective in degrading dilute wastes of endrin (4).

Endrin is metabolized in the rat to give at least 3 metabolites, one of which is 9-ketoendrin (15).



9-Ketoendrin

A 1975 report by Bedford, *et al.* (15), indicates that *syn*-12-hydroxyendrin is rapidly oxidized by the rat *in vivo* (and *in vitro* by liver microsomes) to 12-ketoendrin (which appears to be the compound referred to above as 9-ketoendrin). The isomeric *anti*-12-hydroxyendrin is not so converted and appears as a fecal metabolite in the rat. Rabbits excrete the hydroxyendrins as urinary glucuronides. These findings are significant because all three compounds, the hydroxyendrins and the keto compound, are more toxic than endrin acutely to the rat (16).

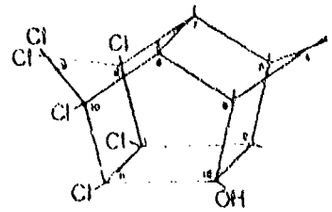
Half-cage ketoendrin (II) and the endrin aldehyde (III) were found as metabolites of both rats and plants (17).

Data from ^{14}C-labeled endrin studies (18) lead to the following conclusions. Most of the endrin is excreted in the feces following a single oral dose to rats, and less than 1% is found in the urine. Of the fecal radioactivity, 70-75% is in the form of hydrophilic metabolites, with the rest as unchanged endrin. Following an intravenous dose, metabolites, but no unchanged endrin, were found in the excreta. Of the hydrophilic metabolites, some 95% appeared to be ketoendrin (II), and the rest was an even more hydrophilic material.

Cole *et al.* (19) gave ^{14}C-tagged endrin intravenously to rats with bile fistulas and found about 50% excreted in the bile within an hour; 90% was eliminated in the feces. Richardson *et al.* (20) fed 0.1 mg/kg/day to dogs for 128 days, and detected blood levels of 0.002-0.008 ppm. At sacrifice, fat samples contained 0.3-0.8 ppm, and liver about 0.08 ppm. From these figures it is clear that endrin is stored poorly in animal tissues, in comparison to other organo-chlorine insecticides.

A review (17) on the metabolism of cyclodiene insecticides collected much information published by mid-1968.

Endrin is apparently broken down in higher plant tissue to endrin ketone (II) and endrin alcohol (V) (21). In cotton (22) some endrin breakdown products may be very soluble in water.



V

ANALYTICAL METHODS

Two colorimetric methods for endrin analysis are available (23, 24). Howeve , dieldrin interferes, giving the same color. The sensitivity of these methods is in the 10-microgram range.

Paper chromatographic separation (25, 26, 27) and thin-layer chromatography (28, 29, 30, 31, 32, 33) have been used for identification of endrin in samples.

Gas chromatography is the best method of analysis. The electron capture (EC) and microcoulometric (MC) detectors are the most sensitive to endrin. A wide variety of conditions have been described for endrin analysis. Table M-3 presents a list of available references dealing with endrin analysis.

Gas chromatographic analysis requires the confirmation of identity. This may be done by determining the retention time of the suspected compound on a second column (34). Better still would be the determination of the mass spectrum of the unknown (35, 36). The mass spectrum of endrin has been published (37). Another method of identity confirmation is chemical conversion to known derivatives BCl_3 catalyzes conversion of endrin to the endrin ketone (38). The same ketone is also generated by heat (3) or UV irradiation (8, 9). Endrin is also converted to a characteristic derivative by the action of $ZnCl_2/HCl$ (39). All of these conversions aid in the gas chromatographic identification of endrin.

TABLE M-3: Gas Chromatographic Analysis of Endrin

Type of Sample	Cleanup of Sample	Detector Used	Limits of Detection	Reference
Water	None	EC	ppt range	40
Blood, eggs, flesh	Extraction only	EC	Not set	41
Wheat (spiked)	Florisil	EC	100 ppb	42
Vegetables	Channel-chromatography	EC	80 ppb	43
Human fat	Florisil	MC	Not set	44
Water (spiked)	None	EC	Not set 10 ppb detected	45
Wastewater	TLC	EC/MC	1.5-22 ppb	46
Lake waters (spiked)	None	EC	5 ppb	47
Lake water, mud, soil	Continuous liquid-liquid extraction	EC	1 ng	48
Water (spiked)	None	EC	0.05 ppb	49
Blood	Extraction only	MC EC	5 ng 2 ng	50
Soil (spiked)	Florisil	EC	Not set 1 ppm detected	51
Potatoes	None	EC	10 ppb	52
Wheat, oats, corn, soybeans	Florisil	EC	10 ppb	53
Water, plants, soil, animal tissues	Silica/gel	EC	Plants & soil-1 ppb Water - 1 ppt Tissues - 4 ppb	54

TABLE M-3: Gas Chromatographic Analysis of Endrin (Cont.)

Type of Sample	Cleanup of Sample	Detector Used	Limits of Detection	Reference
Surface and ground waters (spiked)	Florisil or alumina microcolumns	EC	Not set	55
Fatty vegetables (spiked)	KOH/celite or MgO/celite	EC	Not set, 50 ppb detected	56
None	None	EC	Not set	57
Lake waters (spiked)	Extraction and concentration	EC	1 ppb	58
Broccoli (spiked)	Florisil	MC	2000 ppb	59
Broccoli & lettuce	None	MC	Not set	60
Meat (spiked)	Florisil	EC	Not set, 1000 ppb detected	61
Fish	Florisil	EC	10 ppb	62

HUMAN TOXICOLOGY

Human Exposures

Endrin is considerably more toxic to man (and to other animals as well; see below) than the isomeric dieldrin. It is readily absorbed through the skin, but most poisoning cases have resulted from ingestion. Other characteristics, however, mitigate this acute toxicity: (1) it is not stored in fat or tissues to nearly the extent that dieldrin is and (2) it is very rapidly metabolized. The result, in many instances, is that recovery from severe (but non-lethal) poisoning is quite rapid--days compared to weeks for dieldrin--and blood and tissue levels fall quickly after the peak of intoxication.

Various instances of acute poisoning have led to such estimates of toxic doses as: 0.25 mg/kg could produce a single convulsion; the intake from consuming contaminated bread, 5-50 mg/kg; a lethal dose of 6 grams for man. Concentrations of up to 400 mg/kg in fat and 10 mg/kg in other tissues are said to have been found in fatally poisoned individuals. These and other figures are quoted, with references (18).

More concrete indications of the toxicity of endrin for man come from the reports dealing with outbreaks of poisoning from contaminated flour, in which analyses of the flour in one instance showed endrin concentrations of 200-1500 ppm (63); in another outbreak, with 26 deaths, the bread ranged in concentration from 48-1800 ppm of endrin, made from flour containing 2100-3500 ppm (64). Blood from patients in the 1967 report (64) contained 0.007-0.032 µg/ml of the insecticide.

No studies on human volunteers for chronic endrin toxicity appear to have been done. Jager, 1970 (18) reports no adverse effects in workers engaged in the manufacture of endrin (and who had exposure to aldrin, dieldrin, or telodrin as well). Blood levels of about 0.1 µg/ml were seen only in workers who had accidental spills, and these levels quickly diminished below the detection limit (0.005 µg/ml). Routine biochemical and clinical examinations of these workers failed to show changes that might indicate chronic exposure, except that urinary corticosteroid analyses indicated an increase in 6-β-hydroxycortisol excretion. It was concluded that endrin was responsible for the enzyme induction leading to this increased steroid excretion, although the eight workers examined could have been exposed to other compounds. It was also concluded that in man the toxic threshold for endrin is 0.05-0.1 µg/ml in blood, with a half-life of about 24 hours.

Experimental Animals

Endrin is easily absorbed orally, dermally, or by inhalation. Its vapor pressure, however, is so low that inhalation is not an appreciable risk.

Figures for the acute oral LD_{50} vary, depending, among other variables, on the vehicle used, strain, and age of the animal. For the male rat values of 18-40 mg/kg were found, and for the female 7.5-17 mg/kg; these doses were in oil. For emulsifiable concentrates, values of 4-7 mg/kg were obtained for the rat, with the female showing greater sensitivity (65, 66, 67). Oral LD_{50} values (mg/kg) of 3 for the monkey, 7-10 for rabbits, and 36 for guinea pigs were reported (67). Dermally, with dry endrin, the LD_{50} for the rabbit seemed to be between 125 and 160 mg/kg. Bedford, *et al.* (16) hypothesized that the toxicity of endrin is probably due to the metabolically formed *syn*-12-hydroxyendrin and 12-ketoendrin, the oral LD_{50}'s of these two compounds

being about 1 mg/kg each in male rats compared to an LD_{50} of 5.6 mg/kg for endrin (using dimethylsulfoxide as solvent for the compounds). Female rats were less sensitive to *syn*-12-hydroxyendrin (LD_{50} of 2.8 mg/kg); and *anti*-12-hydroxyendrin (which does not form a ketoendrin) had LD_{50}'s of 2.4 and 5.5 mg/kg for male and female rats, respectively.

A 16-week feeding study (68) showed 60-80% mortality in both sexes in groups receiving 5 ppm of endrin in the diet, and 60% mortality in males receiving 1 ppm. At 100 ppm all rats died by week 4. There was an indication that males were more susceptible than females with respect to mortality. These results are in major disagreement with the more extensive studies next cited (67, 69). Body weight loss and elevated serum alkaline phosphatase values occurred in all groups receiving endrin.

Sub-acute studies as well as a long-term experiment in the rat were performed (67). The sub-acute dosing (six months) indicated that female rats are more susceptible than males to endrin doses of 1-5 mg/kg (in oil) by stomach tube. The two-year dietary feeding study produced increased liver-body weight ratios at 5 ppm, and no change at 1 ppm. Mortality at the two high dose levels (50 and 100 ppm) was almost complete (91% at 106 weeks and 85% at 80 weeks); at 25 ppm and below, mortality was comparable to that of the control group. Of rats that died at 25-100 ppm, diffuse degenerative changes were seen in brain, liver, kidney, and adrenal tissue. Only degenerative changes in the liver were noted in survivors at the higher levels. The tumor incidence was no greater in treated rats than in controls.

In a life-time study in rats given 2, 6, and 12 ppm technical endrin (98%) (69), no appreciable changes were seen in treated rats and the incidence of tumors was comparable to that of controls. The effects of endrin in the dog were also studied (67). The dietary concentrations ranged from 1 to 50 ppm. At levels of 10-50 ppm, the dogs died within 3-6 weeks. Below 10 ppm, the dogs survived for about 18 months. Increased organ-body weight ratios were seen in liver, heart, kidney, and brain, and histopathological changes were noted in the kidneys. Cattle and sheep were not affected by 5 ppm of endrin in their diet for 112 days (13).

In the rat, endrin injected intramuscularly for 45 days at 2 mg/kg daily caused increased blood glucose, presumably from an enhanced synthesis from non-carbohydrate precursors (70). Other observations on the metabolism of endrin are included under the section on "PHYSICAL AND CHEMICAL PROPERTIES".

As indicated above, no evidence of tumorigenicity or carcinogenicity has been found in the long-term studies carried out to date. However, the Working Group of the International Agency for Research on Cancer

has expressed the opinion (71) that the animal data are not sufficient to evaluate the risk of cancer from endrin; and that epidemiological human data do not allow any conclusion to be made in this regard either. The National Cancer Institute in its Division of Cancer Cause and Prevention is currently conducting carcinogenesis studies on endrin. The pesticide is being fed to mice (Strain B6C3FI) and to rats (Strain OM) (72).

The only general reproduction study that seems to have been made on endrin found reduced litter sizes and parent mortality in mice (73). A recent investigation (74) into the teratological effects of endrin (and of aldrin and dieldrin) in mice and golden hamsters showed that cleft palate, open eyes, and webbed feet occurred in the pups following a single oral dose (5 mg/kg) to pregnant animals. These defects often occurred together, as well as a high fetal death rate and growth retardation.

ENVIRONMENTAL CONSIDERATIONS

Behavior in Soil and Water

Persistence: Endrin has very strong adsorptive properties. Lethal quantities to fish may be adsorbed on mud particles, but will not be released into water (75). However, it is degraded more rapidly in flooded soil than in unflooded (76). Biodegradation is aided by fungi and bacteria (e.g., *Trichoderma, Pseudomonas, Bacillus*). The degradation product is ketoendrin (13, 14). *Aerobacter aerogenes* used 55.3% of endrin substrate as its sole carbon source (concentration of 3000 ppm).

Although photolysis and microbial degradation seem to be efficient paths for removal of endrin from soils, endrin has been found to be quite persistent. The estimated half-life for endrin is 4 to 8 years (11). Its disappearance from Congaree sandy loam was variously found to be 59% in 14 years (77), 90% in 16 years (70), and 56% after 13 years (79). In a 16-year study (78), the soil contained 21 ppm endrin, 15 ppm endrin ketone, and 0.4 ppm endrin alcohol.

Leaching and Vaporization: The leaching index indicates less than 10 cm movement in soil given an annual rainfall of 150 cm (80). The vaporization index for endrin is less than 0.1 kg/ha/yr. No field studies on leaching or vaporization are available for endrin; however, the behavior of endrin should parallel that of its isomer dieldrin for which there are field data on leaching (81, 82, 83, 84) and vaporization (85).

Endrin present in river water is stable up to 8 weeks when stored in sealed containers in the laboratory (86). The maximum concentration of endrin found in a U.S. surface water through 1968 was 0.133 ppb (87).

Only 3 out of 20 agricultural soils surveyed in Northeastern Saskatchewan contained endrin at the level of 10-20 ppb. All other soils were free of endrin or endrin was below a detectable limit of 10 ppb (88).

Animals

Mammals: For information on the behavior of endrin in mammals, see HUMAN TOXICOLOGY-*Experimental Animals*.

Birds: Endrin is lethal to pheasants fed 5 ppm in food over a period of time. It is not toxic to quail at 1 ppm in feed (89).

Fish: Fish are extremely sensitive to endrin, e.g., 96-hour LD_{50}'s are as follows: bluegill, 0.6 ppb; bluntnose minnow and coho salmon, 0.27 ppb; goldfish, 1.96 ppb; and fathead minnow, 1.8 ppb (90). Eggs and fry are less susceptible than adult fish (91) and toxicity is reduced at lower temperatures (92, 93).

A 3-week exposure of spot (*Leiostomus xanthurus*) produced systemic lesions involving brain and spinal cord, liver, kidneys, and stomach (94). Exposure of cutthroat trout (*Salmo clarkii*) to endrin levels ranging from 1 to 40 ppb for 30 minutes each 4 weeks over a 1-year period, produced pathological changes in the gill, liver, pancreas, brain, and gonad. Hepatic lesions in young trout were of a type frequently described as preceding the development of hepatomas in nutritionally deficient fish. The increased incidence and severity of hepatic degenerative changes observed in fish exposed to high levels of endrin suggested that nutritional deficiency is associated with exposure to endrin (95).

Invertebrates: Numbers of soil collembola, earthworms, mites, and millipedes were decreased as a result of normal endrin application. Earthworms concentrated endrin by a factor of 3.6 as compared with ambient soil; slugs contained 10.3 ppm after normal application in a black currant nursery (96).

Microorganisms: Soil microorganisms are relatively resistant to endrin. No effect was noted on total numbers of bacteria and fungi after 5 annual applications of 5 lbs/acre (97).

Degradation: Patil, *et al.* (13), isolated 20 microorganisms capable of degrading dieldrin and attempted, successfully, to grow them on 10^{-6} *M* endrin. Specifically, strains of *Trichomonas viride*, *Pseudomonas* sp., *Micrococcus* sp., *Arthrobacter* sp., and *Bacillus* sp. could reproduce at that concentration of endrin. Matsumura, et al. (14) found 25 endrin-active isolates from 150 soil samples. They found 7 metabolites, including 3 major ones (one was keto-endrin) from a culture of *Pseudomonas*.

In a radioisotope study, Patil, *et al.* (4), exposed samples of marine sediment microflora to 0.1 µmole ^{14}C-endrin. Two metabolic products were found in positive cultures, one was identified as keto-endrin.

Plants

Phytotoxicity: Endrin injures *Dianthus, Kalanchoe, Lilium longiflorum, Philodendron* and scabious, as evidenced by retarded growth, inhibited flowering, and leaf burn (application of 6 pounds endrin per acre for *Dianthus*, and 2 pounds endrin per acre for scabious) (98). Kerr and Kuitert (99) report that high concentrations of endrin will injure Easter lilies (endrin treatment of 1 pint 18% emulsifiable concentrate per acre). Endrin in soil has been shown to reduce dry matter yield of wheat growing on the soil and alter nitrogen and phosphorus uptake in wheat and corn (100). Increased phytotoxicity of endrin may come in synergistic or additive effects with other insecticides (99).

Bioaccumulation: Endrin may contaminate plants through root absorption, through leaf absorption, and by contact of foliage with dust particles (101. Soybeans growing in soil containing 20 ppm endrin accumulated endrin through foliar absorption of endrin vapor and through root absorption from soil with translocation into aerial parts (Table M-4) (101).

TABLE M-4: Concentration of Residues Found in Soybean Plants Exposed to Soil Treated with Endrin (20 ppm) (101).

Plant Part	Endrin Accumulation (ppm)*	
	Vapor Sorption	Root Sorption
Upper leaves	15.95	87.71
Lower leaves	33.78	160.55
Upper stem	2.42	217.30
Lower stem	1.66	359.62
Pods	2.84	10.44
Seeds	0.99	3.30

*Based on ^{14}C content of combusted dry plant parts.

On a whole plant basis, root absorption of endrin is over 5 times greater than vapor absorption (101).

Endrin is reportedly absorbed by roots of plants to the same degree as dieldrin (101). Endrin residues have been found in peanuts, radishes, carrots, sugarbeets, potatoes, turnips, oats, wheat, soybeans, cotton, corn, alfalfa, bromegrass and cucumbers (22).

Uptake through root absorption appears to be continuous throughout most of a plant's (demonstrated with soybeans) growth period (102). Root crops accumulate endrin in their edible fractions. Carrots and radishes grown in soil containing 3.91 ppm endrin before planting accumulated 0.06 ppm and 0.04 ppm, respectively (103). No detectable amounts of endrin were found in turnips or onions growing on the same soil.

Soil type influences endrin uptake by plants. Studies on 5 types of soils demonstrated uptake of endrin by seedlings of soybean, wheat, corn, alfalfa, bromegrass, and cucumber (104). Application of 5 ppm to soils led to endrin residues in the plant tissue of up to 0.594 ppm for soybeans, 2.241 ppm for wheat, 1.132 ppm for corn, 5.989 ppm for alfalfa, 6.225 ppm for bromegrass, and 1.734 ppm for cucumbers. For all plants, greatest uptake was from a Lakeland sandy loam and Sharkey clay. High silt proportions in the soil decrease endrin uptake, but organic matter in the soil appears to have no effect (104). The amount of endrin residue accumulated by plants is also related to the amount of endrin in the soil. Studies with wheat and soybean seedlings grown in soil containing up to 5 ppm endrin demonstrated that residues in the wheat were about 50% of the initial concentration in the soil and that residues in soybeans are about 10% the initial concentration in the soil (101).

Degradation: See section on "PHYSICAL AND CHEMICAL PROPERTIES."

Food Chain

One study with a model ecosystem was carried out using ^{14}C-endrin (105). The endrin was applied at a level equivalent to 1 lb/acre. After 63 days, the distribution of endrin was determined in the organisms in the model system. This distribution is presented in Table M-5.

TABLE M-5: Transport of Endrin Through a Food Chain in a Model Ecosystem (105).

	Endrin (ppm)			
	H_2O	Algae	Snail	Fish
Total C^{14}	0.0134	13.62	150.58	4.48
Endrin	0.0025	11.56	125.00	3.40
Sum of 4 Unknowns	0.00639	2.06	20.18	1.08

The milk of cattle experimentally fed endrin contained endrin residues and the relationship was established between the residues in milk and the levels of endrin in the cows' diet (106). However, in 1957 and 1960 the Food and Drug Administration found no significant residues of any cyclodiene insecticides in milk (106).

Analysis of human adipose tissue samples showed no endrin, whereas 45% of these samples contained 0.1-0.4 ppm of dieldrin (44).

EXISTING STANDARDS

Existing standards for endrin are: threshold limits volume (TLV) of 0.1 mg/m^3 (107); drinking water standard of 0.0002 ppm (108); and acceptable daily intake (ADI) of 0.0002 mg/kg/day (109).

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